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<b>(21) International Application Number:</b> PCT/US98/08542 <b>(22) International Filing Date:</b> 28 April 1998 (28.04.98)  <b>(30) Priority Data:</b> 08/846,432 30 April 1997 (30.04.97) US  <b>(71) Applicant:</b> MOLECUMETICS LTD. [US/US]; Suite 400, 2023 - 120th Avenue N.E., Bellevue, WA 98005 (US).  <b>(72) Inventors:</b> KAHN, Michael, S.; 10916 - 80th Place Northeast, Kirkland, WA 98034 (US). EGUCHI, Masakatsu; 636 - 129th Place Northeast, Bellevue, WA 98005 (US). KIM, Hwa-Ok; 5606 - 154th Avenue Northeast, Redmond, WA 98052 (US).  <b>(74) Agents:</b> HERMANNS, Karl, R. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).		<b>(81) Designated States:</b> AU, CA, JP, KR, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title:</b> REVERSE-TURN MIMETICS AND METHODS RELATING THERETO  <b>(57) Abstract</b>  Conformationally constrained compounds which mimic the secondary structure of reverse-turn regions of biologically active peptides and proteins are disclosed. Such reverse-turn mimetics have utility over a wide range of fields, including use as diagnostic and therapeutic agents. Libraries containing the reverse-turn mimetics of this invention are also disclosed, as well as methods for screening the same to identify biologically active members.		

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Description

## REVERSE-TURN MIMETICS AND METHODS RELATING THERETO

5 Technical Field

The present invention relates generally to reverse-turn mimetics and to a chemical library of reverse-turn mimetics.

10 Background of the Invention

Random screening of molecules for possible activity as therapeutic agents has occurred for many years and resulted in a number of important drug discoveries. While advances in molecular biology and computational chemistry have led to increased interest in what has been termed "rational drug design," such techniques have not proven as fast or reliable as initially predicted. Thus, in recent years there has been a renewed interest and return to random drug screening. To this end, particular strides having been made in new technologies based on the development of combinatorial chemistry libraries, and the screening of such libraries in search for biologically active members.

In general, combinatorial chemistry libraries are simply a collection of molecules. Such libraries vary by the chemical species within the library, as well as the methods employed to both generate the library members and identify which members interact with biological targets of interest. While this field is still young, methods for generating and screening libraries have already become quite diverse and sophisticated. For example, a recent review of various combinatorial chemical libraries has identified a number of such techniques, including the use of both tagged and untagged library members (Janda, Proc. Natl. Acad. Sci. USA 91:10779-10785, 1994).

To date, combinatorial chemistry libraries have generally been limited to members of peptide or nucleotide origin. To this end, the techniques of Houghten et al. illustrate an example of what is term a "dual-defined  
5 iterative" method to assemble soluble combinatorial peptide libraries via split synthesis techniques (*Nature (London)* 354:84-86, 1991; *Biotechniques* 13:412-421, 1992; *Bioorg. Med. Chem. Lett.* 3:405-412, 1993). By this technique, soluble peptide libraries containing tens of  
10 millions of members have been obtained. Such libraries have been shown to be effective in the identification of opioid peptides, such as methionine- and leucine-enkephalin (Dooley and Houghten, *Life Sci.* 52, 1509-1517, 1993), and a N-acylated peptide library has been used to  
15 identify acetalins, which are potent opioid antagonists (Dooley et al., *Proc. Natl. Acad. Sci. USA* 90:10811-10815, 1993. More recently, an all D-amino acid opioid peptide library has been constructed and screened for analgesic activity against the mu (" $\mu$ ") opioid receptor (Dooley  
20 et al, *Science* 266:2019-2022, 1994).

While combinatorial libraries containing members of peptide and nucleotide origin are of significant value, there is still a need in the art for libraries containing members of different origin. For example, traditional  
25 peptide libraries to a large extent merely vary the amino acid sequence to generate library members. While it is well recognized that the secondary structures of peptides are important to biological activity, such peptide libraries do not impart a constrained secondary structure  
30 to its library members.

To this end, some researchers have cyclized peptides with disulfide bridges in an attempt to provide a more constrained secondary structure (Tumelty et al., *J. Chem. Soc.* 1067-68, 1994; Eichler et al., *Peptide Res.*  
35 7:300-306, 1994). However, such cyclized peptides are

generally still quite flexible and are poorly bioavailable, and thus have met with only limited success.

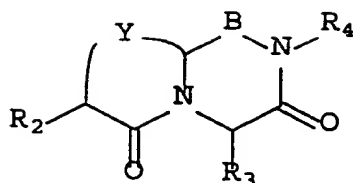
More recently, non-peptide compounds have been developed which more closely mimic the secondary structure of reverse-turns found in biologically active proteins or peptides. For example, U.S. Patent No. 5,440,013 to Kahn and published PCT WO94/03494 to Kahn both disclose conformationally constrained, non-peptidic compounds which mimic the three-dimensional structure of reverse-turns.

While significant advances have been made in the synthesis and identification of conformationally constrained, reverse-turn mimetics, there is still a need in the art for small molecules which mimic the secondary structure of peptides. There is also a need in the art for libraries containing such members, as well as techniques for synthesizing and screening the library members against targets of interest, particularly biological targets, to identify bioactive library members. The present invention fulfills these needs, and provides further related advantages.

#### Summary of the Invention

In brief, the present invention is directed to conformationally constrained compounds which mimic the secondary structure of reverse-turn regions of biologically active peptides and proteins. This invention also discloses libraries containing such compounds, as well as the synthesis and screening thereof.

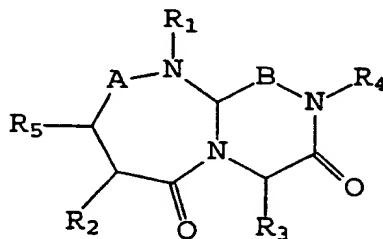
The compounds of the present invention have the following general structure (I):



(I)

wherein Y is selected from -CH(R<sub>5</sub>)-A-N(R<sub>1</sub>)-, -A-N(R<sub>1</sub>)-CH(R')-, -A-N(R<sub>1</sub>)-C(=O)-, -A-C(=O)-N(R<sub>1</sub>)-, -A-CH(R<sub>1</sub>)-O-, and -A-CH(R<sub>1</sub>)-N(R')-; A is -(CHR')<sub>n</sub>-; B is -(CHR'')<sub>m</sub>-; n = 0, 1 or 2; m = 1, 2 or 3; and any two adjacent CH groups or adjacent NH and CH groups on the bicyclic ring may optionally form a double bond; and wherein R', R'', R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are as defined in the following detailed description.

In the embodiment wherein Y is -CH(R<sub>5</sub>)-A-N(R<sub>1</sub>)-, the compounds of this invention have the following structure (I'):

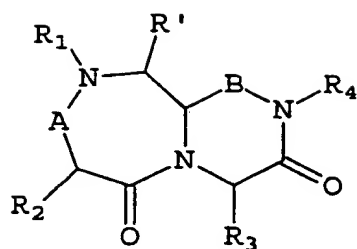


(I')

wherein A and B are as defined above, and R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are as defined in the following detailed description.

In the embodiment wherein Y is -A-N(R<sub>1</sub>)-CH(R')-, the compounds of this invention have the following structure (I''):

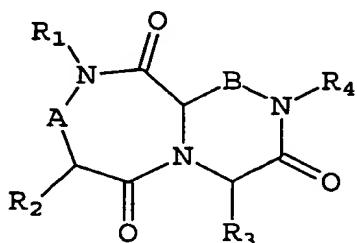
5



(I'')

wherein A and B are as defined above, and R', R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as defined in the following detailed description.

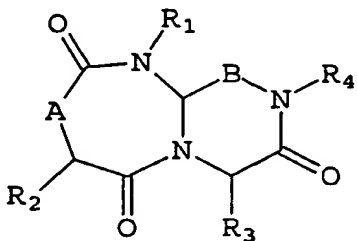
In the embodiment wherein Y is -A-N(R<sub>1</sub>)-C(=O)-, the compounds of this invention have the following structure (I'''):



(I''')

wherein A and B are as defined above, and R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as defined in the following detailed description.

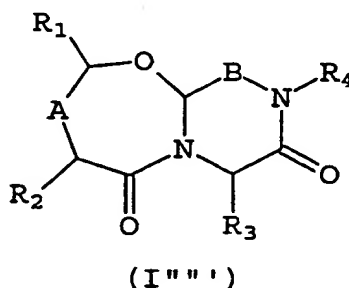
In the embodiment wherein Y is -A-C(=O)-N(R<sub>1</sub>)-, the compounds of this invention have the following structure (I'''):



(I''')

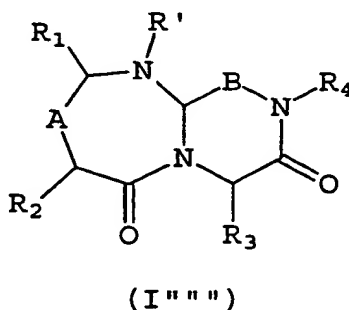
wherein A and B are as defined above, and  $R_1$ ,  $R_2$ ,  $R_3$ , and  $R_4$  are as defined in the following detailed description.

In the embodiment wherein Y is  $-A-CH(R_1)-O-$ , the compounds of this invention have the following structure (I'''):



wherein A and B are as defined above, and  $R_1$ ,  $R_2$ ,  $R_3$ , and  $R_4$  are as defined in the following detailed description.

In the embodiment wherein Y is  $-A-CH(R_1)-N(R')-$ , the compounds of this invention have the following structure (I''):



wherein A and B are as defined above, and  $R'$ ,  $R_1$ ,  $R_2$ ,  $R_3$ , and  $R_4$  are as defined in the following detailed description.

The present invention is also directed to libraries containing compounds of structure (I) above, as well as methods for synthesizing such libraries and methods for screening the same to identify biologically active compounds. Compositions containing a compound of



this invention in combination with a pharmaceutically acceptable carrier or diluent are also disclosed.

These and other aspects of this invention will be apparent upon reference to the attached figures and following detailed description. To this end, various references are set forth herein which describe in more detail certain procedures, compounds and/or compositions, and are incorporated by reference in their entirety.

10 Brief Description of the Drawing

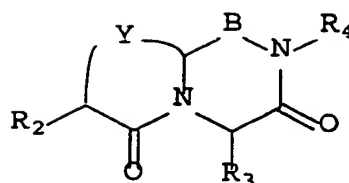
Figure 1 illustrates the percent inhibition of radioligand binding to  $\delta$  and  $\mu$  opiate receptors of a representative reverse-turn mimetic of this invention as a function of concentration.

15 Figures 2-8 illustrate representative reaction schemes for the synthesis of reverse-turn mimetics of this invention.

Detailed Description of the Invention

20 The present invention is directed to reverse-turn mimetics and chemical libraries containing reverse-turn mimetics. The reverse-turn mimetics of the present invention are useful as bioactive agents, including (but not limited to) use as diagnostic, prophylactic and/or therapeutic agents. The reverse-turn mimetic libraries of this invention are useful in the identification of such bioactive agents. In the practice of the present invention, the libraries may contain from tens to hundreds to thousands (or greater) of individual reverse-turn  
25 mimetics (also referred to herein as "members").

30 In one aspect of the present invention, a reverse-turn mimetic is disclosed having the following structure (I):



(I)

wherein Y is selected from -CH(R<sub>5</sub>)-A-N(R<sub>1</sub>)-, -A-N(R<sub>1</sub>)-CH(R')-, -A-N(R<sub>1</sub>)-C(=O)-, -A-C(=O)-N(R<sub>1</sub>)-, -A-CH(R<sub>1</sub>)-O- and -A-CH(R<sub>1</sub>)-N(R')-; A is -(CHR')<sub>n</sub>-; B is -(CHR'')<sub>m</sub>-; n = 0, 1 or 2; m = 1, 2 or 3; and any two adjacent CH groups or adjacent NH and CH groups on the bicyclic ring may optionally form a double bond; and wherein R', R'', R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are as defined below.

In structures (I') through (I''') above a solid line designation for attachment of the various R groups to a carbon atom on the fused bicyclic ring indicates that these R groups may lie either above or below the plane of the page. If a reverse-turn mimetic of this invention is intended to mimic a reverse-turn of naturally occurring amino acids (i.e., "L-amino acids"), the R groups would generally lie below the plane of the page (i.e., "⋈ R") in Structure (I). However, if the reverse-turn mimetic of this invention is intended to mimic a reverse-turn containing one or more D-amino acids, then the corresponding R group or groups would lie above the plane of the page (i.e., "— R") in Structure (I).

In one embodiment, R<sub>1</sub> and R<sub>4</sub> are the same or different and represent the remainder of the compound, and R', R'', R<sub>2</sub>, R<sub>3</sub>, and R<sub>5</sub> are the same or different and independently selected from an amino acid side chain moiety or derivative thereof. With regard to R' and R'', it should be understood that each occurrence of R' and R'' is independently selected from amino acid side chain moieties or derivatives thereof. For example, when m=2, B is a -CHR''CHR''- moiety. In this instance, both

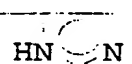
occurrences of R" are independently selected, and may be the same or different. Thus, if the first occurrence of R" is hydrogen and the second methyl, B would have the structure  $-\text{CH}_2\text{CH}(\text{CH}_3)-$ .

5 As used herein, the term "remainder of the compound" means any moiety, agent, compound, support, molecule, linker, amino acid, peptide or protein covalently attached to the reverse-turn mimetic at either the R<sub>1</sub> and/or R<sub>4</sub> positions. This term also includes amino  
10 acid side chain moieties and derivatives thereof.

As used herein, the term "amino acid side chain moiety" represents any amino acid side chain moiety present in naturally occurring proteins including (but not limited to) the naturally occurring amino acid side chain  
15 moieties identified in Table 1. Other naturally occurring amino acid side chain moieties of this invention include (but are not limited to) the side chain moieties of 3,5-dibromotyrosine, 3,5-diiodotyrosine, hydroxylysine,  $\gamma$ -carboxyglutamate, phosphotyrosine and phosphoserine. In  
20 addition, glycosylated amino acid side chains may also be used in the practice of this invention, including (but not limited to) glycosylated threonine, serine and asparagine.

Table 1

25 Amino Acid Side Chain Moieties

<u>Amino Acid Side Chain Moiety</u>	<u>Amino Acid</u>
-H	Glycine
-CH <sub>3</sub>	Alanine
-CH(CH <sub>3</sub> ) <sub>2</sub>	Valine
-CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	Leucine
-CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	Isoleucine
-(CH <sub>2</sub> ) <sub>4</sub> NH <sub>3</sub> <sup>+</sup>	Lysine
-(CH <sub>2</sub> ) <sub>3</sub> NHC(NH <sub>2</sub> )NH <sub>2</sub> <sup>+</sup>	Arginine
-CH <sub>2</sub> - 	Histidine

$-\text{CH}_2\text{COO}^-$	Aspartic acid
$-\text{CH}_2\text{CH}_2\text{COO}^-$	Glutamic acid
$-\text{CH}_2\text{CONH}_2$	Asparagine
$-\text{CH}_2\text{CH}_2\text{CONH}_2$	Glutamine
$-\text{CH}_2-\text{C}_6\text{H}_5$	Phenylalanine
$-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$	Tyrosine
$-\text{CH}-\text{C}_8\text{H}_6\text{N}_2$	Tryptophan
$-\text{CH}_2\text{SH}$	Cysteine
$-\text{CH}_2\text{CH}_2\text{SCH}_3$	Methionine
$-\text{CH}_2\text{OH}$	Serine
$-\text{CH}(\text{OH})\text{CH}_3$	Threonine
$-\text{HN}-\text{C}_4\text{H}_7$	Proline
$-\text{HN}-\text{C}_4\text{H}_6\text{OH}$	Hydroxyproline

In addition to naturally occurring amino acid side chain moieties, the amino acid side chain moieties of the present invention also include various derivatives thereof. As used herein, a "derivative" of an amino acid side chain moiety includes modifications and/or variations to naturally occurring amino acid side chain moieties. For example, the amino acid side chain moieties of alanine, valine, leucine, isoleucine and phenylalanine may generally be classified as lower chain alkyl, aryl, or aralkyl moieties. Derivatives of amino acid side chain moieties include other straight chain or branched, cyclic or noncyclic, substituted or unsubstituted, saturated or unsaturated lower chain alkyl, aryl or aralkyl moieties.

As used herein, "lower chain alkyl moieties" contain from 1-12 carbon atoms, "lower chain aryl moieties" contain from 6-12 carbon atoms and "lower chain

aralkyl moieties" contain from 7-12 carbon atoms. Thus, in one embodiment, the amino acid side chain derivative is selected from a C<sub>1-12</sub> alkyl, a C<sub>6-12</sub> aryl and a C<sub>7-12</sub> aralkyl, and in a more preferred embodiment, from a C<sub>1-7</sub> alkyl, a  
5 C<sub>6-10</sub> aryl and a C<sub>7-11</sub> aralkyl.

Amino side chain derivatives of this invention further include substituted derivatives of lower chain alkyl, aryl, and aralkyl moieties, wherein the substituent is selected from (but are not limited to) one or more of  
10 the following chemical moieties: -OH, -OR, -COOH, -COOR, -CONH<sub>2</sub>, -NH<sub>2</sub>, -NHR, -NRR, -SH, -SR, -SO<sub>2</sub>R, -SO<sub>2</sub>H, -SOR and halogen (including F, Cl, Br and I), wherein each occurrence of R is independently selected from straight chain or branched, cyclic or noncyclic, substituted or  
15 unsubstituted, saturated or unsaturated lower chain alkyl, aryl and aralkyl moieties. Moreover, cyclic lower chain alkyl, aryl and aralkyl moieties of this invention include naphthalene, as well as heterocyclic compounds such as thiophene, pyrrole, furan, imidazole, oxazole, thiazole,  
20 pyrazole, 3-pyrroline, pyrrolidine, pyridine, pyrimidine, purine, quinoline, isoquinoline and carbazole. Amino acid side chain derivatives further include heteroalkyl derivatives of the alkyl portion of the lower chain alkyl and aralkyl moieties, including (but not limited to) alkyl  
25 and aralkyl phosphonates and silanes.

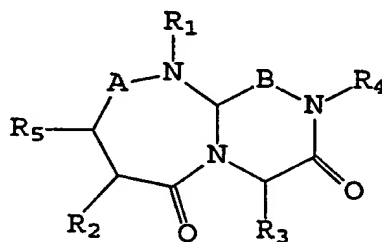
Representative R<sub>1</sub> and R<sub>4</sub> moieties specifically include (but are not limited to) -OH, -OR, -COR, -COOR, -CONH<sub>2</sub>, -CONR, -CONRR, -NH<sub>2</sub>, -NHR, -NRR, -SO<sub>2</sub>R and -COSR, wherein each occurrence of R is as defined above.

30 In a further embodiment, and in addition to being an amino acid side chain moiety or derivative thereof (or the remainder of the compound in the case of R<sub>1</sub> and R<sub>4</sub>), R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, or R<sub>5</sub> may be a linker facilitating the linkage of the compound to another moiety or compound.  
35 For example, the compounds of this invention may be linked to one or more known compounds, such as biotin, for use in

diagnostic or screening assay. Furthermore,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  or  $R_5$  may be a linker joining the compound to a solid support (such as a support used in solid phase peptide synthesis) or alternatively, may be the support itself.

5 In this embodiment, linkage to another moiety or compound, or to a solid support, is preferable at the  $R_1$  or  $R_4$  position, and more preferably at the  $R_4$  position.

In the embodiment where Y is  $-\text{CH}(R_5)-\text{A}-\text{N}(R_1)-$ , the reverse-turn mimetic has the following structure (I'):

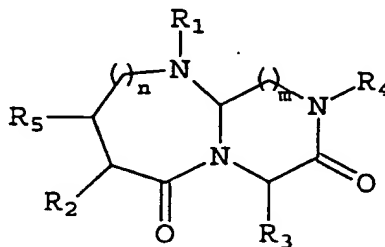


(I')

wherein A, B,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are as defined above. In a preferred embodiment,  $R_1$  and  $R_4$  represent the remainder of the compound, and  $R_2$ ,  $R_3$  and  $R_5$  are

15 individually selected from an amino acid side chain moiety.

In a more specific embodiment of structure (I'), A is  $-(\text{CH}_2)_n-$ , B is  $-(\text{CH}_2)_m-$ , and the reverse-turn mimetic has the following structure (Ia'):

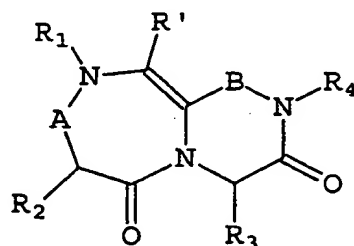


(Ia')

wherein  $n$ ,  $m$ ,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  are as defined above.

In the embodiment where Y is  $-A-N(R_1)-CH(R')-$ , and two adjacent CH groups on the bicyclic ring form a double bond, the reverse-turn mimetics of this invention include the following structure (Ia''):

5

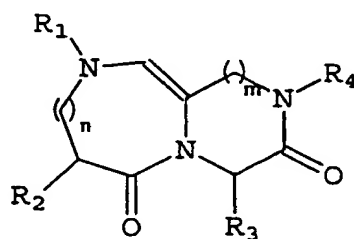


(Ia'')

wherein A, B,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R'$  are as defined above. In a preferred embodiment,  $R_1$  and  $R_4$  represent the remainder of the compound,  $R_2$  and  $R_3$  are independently selected from an amino acid side chain moiety, and  $R'$  is hydrogen.

In a more specific embodiment of structure (Ia''), A is  $-(CH_2)_n-$ , B is  $-(CH_2)_m-$ ,  $R'$  is hydrogen, and the reverse-turn mimetic has the following structure (Ib''):

15



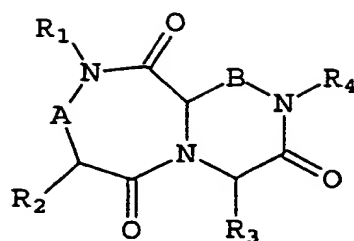
(Ib'')

wherein  $n$ ,  $m$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above.

20

In the embodiment where Y is  $-A-N(R_1)-C(=O)-$ , the reverse turn mimetic has the following structure (I'''):

14

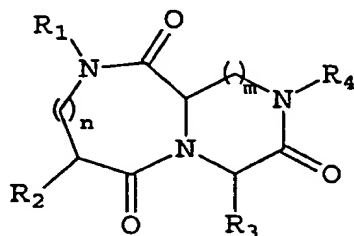


(I'')

wherein A, B, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as defined above. In a preferred embodiment, R<sub>1</sub> and R<sub>4</sub> represent the remainder of the compound, and R<sub>2</sub> and R<sub>3</sub> are independently selected from an amino acid side chain moiety.

In a more specific embodiment of structure (I''), A is -(CH<sub>2</sub>)<sub>n</sub>-, B is -(CH<sub>2</sub>)<sub>m</sub>-, and the reverse-turn mimetic has the following structure (Ia''):

10

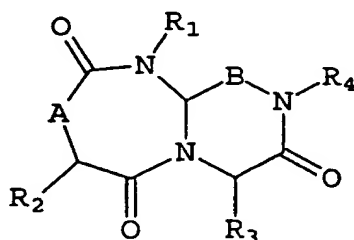


(Ia'')

wherein n, m, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as defined above.

In the embodiment where Y is -A-C(=O)-N(R<sub>1</sub>)-, the reverse turn mimetic has the following structure (I'''):

15



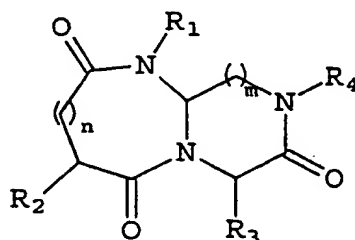
(I''')

wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are as defined above. In a preferred embodiment, R<sub>1</sub> and R<sub>4</sub> represent the remainder of the compound, and R<sub>2</sub> and R<sub>3</sub> are independently selected from an amino acid side chain moiety.

20



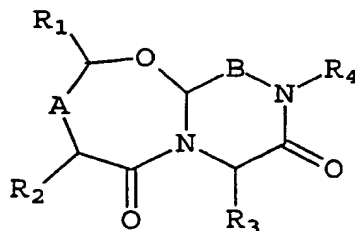
In a more specific embodiment of structure (I'''), A is  $-(CH_2)_n-$ , B is  $-(CH_2)_m-$ , and the reverse-turn mimetic has the following structure (Ia'''):



(Ia''')

wherein  $n$ ,  $m$ ,  $R_1$ ,  $R_2$ ,  $R_3$ , and  $R_4$  are as defined above.

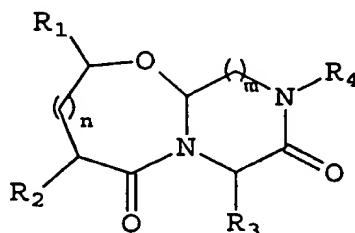
In the embodiment where Y is  $-A-CH(R_1)-O-$ , the reverse-turn mimetic has the following structure (I'''):



(I''')

wherein  $R_1$ ,  $R_2$ ,  $R_3$ , and  $R_4$  are as defined above. In a preferred embodiment,  $R_1$  and  $R_4$  represent the remainder of the compound, and  $R_2$  and  $R_3$  are independently selected from an amino acid side chain moiety.

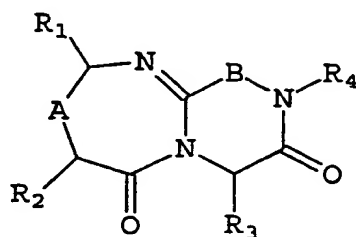
In a more specific embodiment of structure (I'''), A is  $-(CH_2)_n-$ , B is  $-(CH_2)_m-$ , and the reverse-turn mimetic has the following structure (Ia'''):



(Ia''')

wherein  $n$ ,  $m$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above.

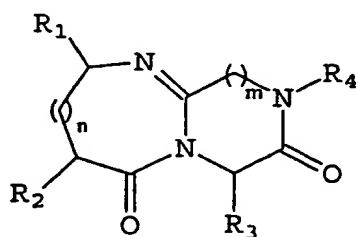
In the embodiment where  $Y$  is  $-A-CH(R_1)-N(R')-$ , and adjacent NH and CH groups on the bicyclic ring form a double bond, the reverse-turn mimetics of this invention include the following structure (Ia''):



(Ia'')

wherein  $A$ ,  $B$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above. In a preferred embodiment,  $R_1$  and  $R_4$  represent the remainder of the compound, and  $R_2$  and  $R_3$  are independently selected from an amino acid side chain moiety.

In a more specific embodiment of structure (Ia''),  $A$  is  $-(CH_2)_n-$ ,  $B$  is  $-(CH_2)_m-$ , and the reverse-turn mimetic has the following structure (Ib''):



(Ib'')

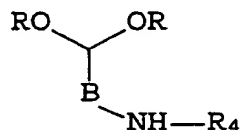
wherein  $n$ ,  $m$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above.

The reverse-turn mimetics of the present invention may be prepared by utilizing appropriate starting component molecules (hereinafter referred to as "component pieces"). Briefly, in the synthesis of reverse turn mimetics having structure (I'), first and second component pieces are coupled to form a combined first-second intermediate, third and fourth component pieces are

coupled to form a combined third-fourth intermediate (or, if commercially available, a single third intermediate may be used), the combined first-second intermediate and third-fourth intermediate (or third intermediate) are then  
5 coupled to provide a first-second-third-fourth intermediate (or first-second-third intermediate) which is cyclized to yield the reverse-turn mimetics of this invention. Alternatively, the reverse-turn mimetics of structure (I') may be prepared by sequential coupling of  
10 the individual component pieces either stepwise in solution or by solid phase synthesis as commonly practiced in solid phase peptide synthesis.

Within the context of the present invention, a "first component piece" has the following structure 1:

15

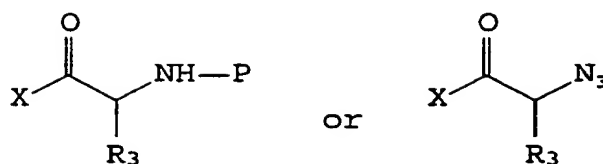


1

where  $\text{R}_4$  and B are as defined above, and R is a protective group suitable for use in peptide synthesis. Suitable R groups include alkyl groups and, in a preferred  
20 embodiment, R is a methyl group. Such first component pieces may be readily synthesized by reductive amination by mating  $\text{CH}(\text{OR})_2-(\text{CH}_2)_m\text{-CHO}$  with  $\text{H}_2\text{N}-\text{R}_4$ , or by displacement from  $\text{CH}(\text{OR})_2-(\text{CH}_2)_m\text{-Br}$ .

A "second component piece" of this invention has  
25 the following structure 2:

18



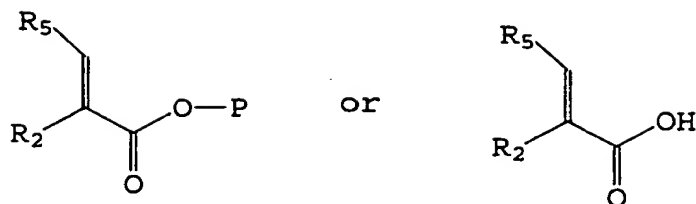
2

where  $R_3$  is as defined above, P is an amino protective group suitable for use in peptide synthesis, and X represents the leaving group of the activated carboxylic acid group. Preferred protective groups include t-butyl dimethylsilyl (TBDMS), BOC, FMOC, and Alloc (allyloxycarbonyl). N-Protected amino acids are commercially available. For example, FMOC amino acids are available from a variety of sources. The conversion of these compounds to the second component pieces of this invention may be readily achieved by activation of the carboxylic acid group of the N-protected amino acid. Suitable activated carboxylic acid groups include acid halides where X is a halide such as chloride or bromide, acid anhydrides where X is an acyl group such as acetyl, reactive esters such as an N-hydroxysuccinimide esters and pentafluorophenyl esters, and other activated intermediates such as the active intermediate formed in a coupling reaction using a carbodiimide such as dicyclohexylcarbodiimide (DCC).

In the case of the azido derivative of an amino acid serving as the second component piece, such compounds may be prepared from the corresponding amino acid by the reaction disclosed by Zaloom et al. (*J. Org. Chem.* 46:5173-76, 1981).

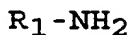
A "third component piece" of this invention has the following structure 3:

19

3

where  $R_2$  and  $R_5$  are as defined above, and P is a carboxylic acid protective group such as a methyl or t-butyl group.

5 A "fourth component piece" of this invention has the following structure 4:



10

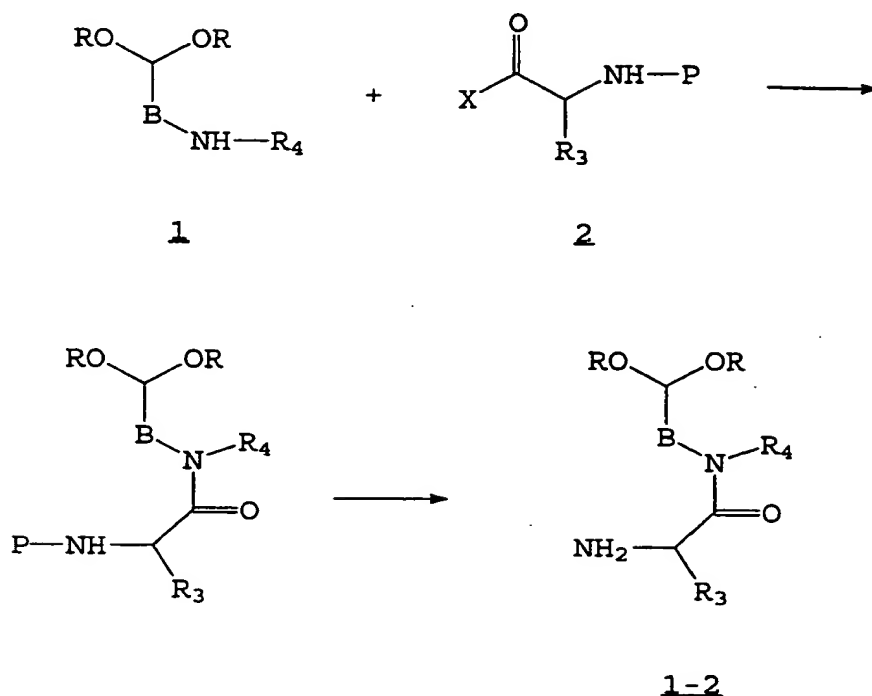
4

where  $R_1$  is as defined above. Suitable fourth component pieces are commercially available from a variety of sources. Alternatively, the fourth component pieces may  
15 be readily prepared by standard organic synthetic techniques commonly utilized for the synthesis of primary amines.

More specifically, the reverse-turn mimetics of this invention of structure (I') are synthesized by  
20 reacting a first component piece with a second component piece to yield a combined first-second intermediate, followed by either reacting the combined first-second intermediate with third and fourth component pieces sequentially, or reacting the intermediate with a combined  
25 third-fourth intermediate to provide a combined first-second-third-fourth intermediate, and then cyclizing this intermediate to yield the reverse-turn mimetic.

The general synthesis of a reverse-turn mimetic having structure I' may be synthesized by the following  
30 technique. A first component piece 1 is coupled to a

second component piece 2 to yield, after N-deprotection, a combined first-second intermediate 1-2 as illustrated below:

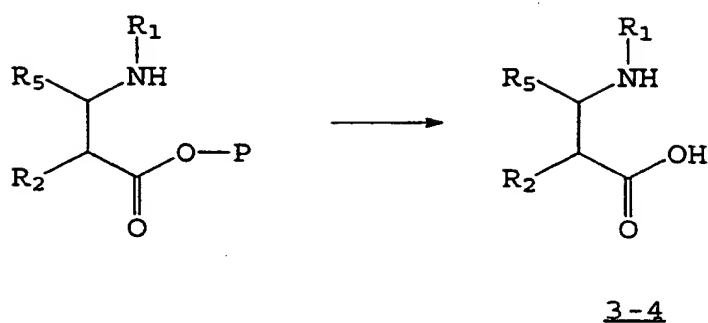
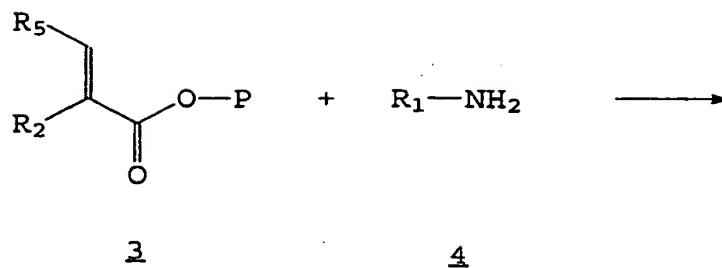


5

The synthesis of the reverse-turn mimetic may be convergent, in which case a combined third-fourth intermediate 3-4 is prepared from the coupling of a third component piece 3 with a fourth component piece 4 to yield, after O-deprotection, a combined third-fourth intermediate 3-4 as illustrated below:

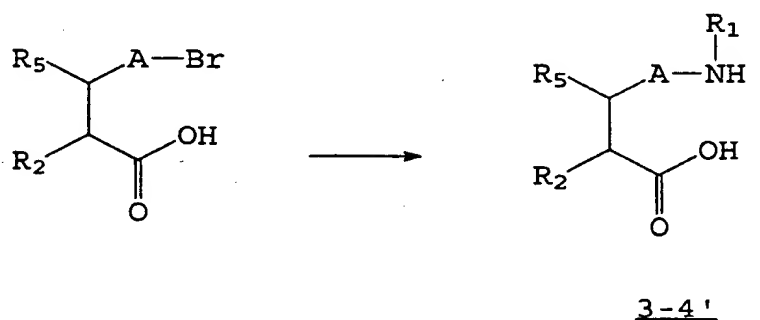
10

21



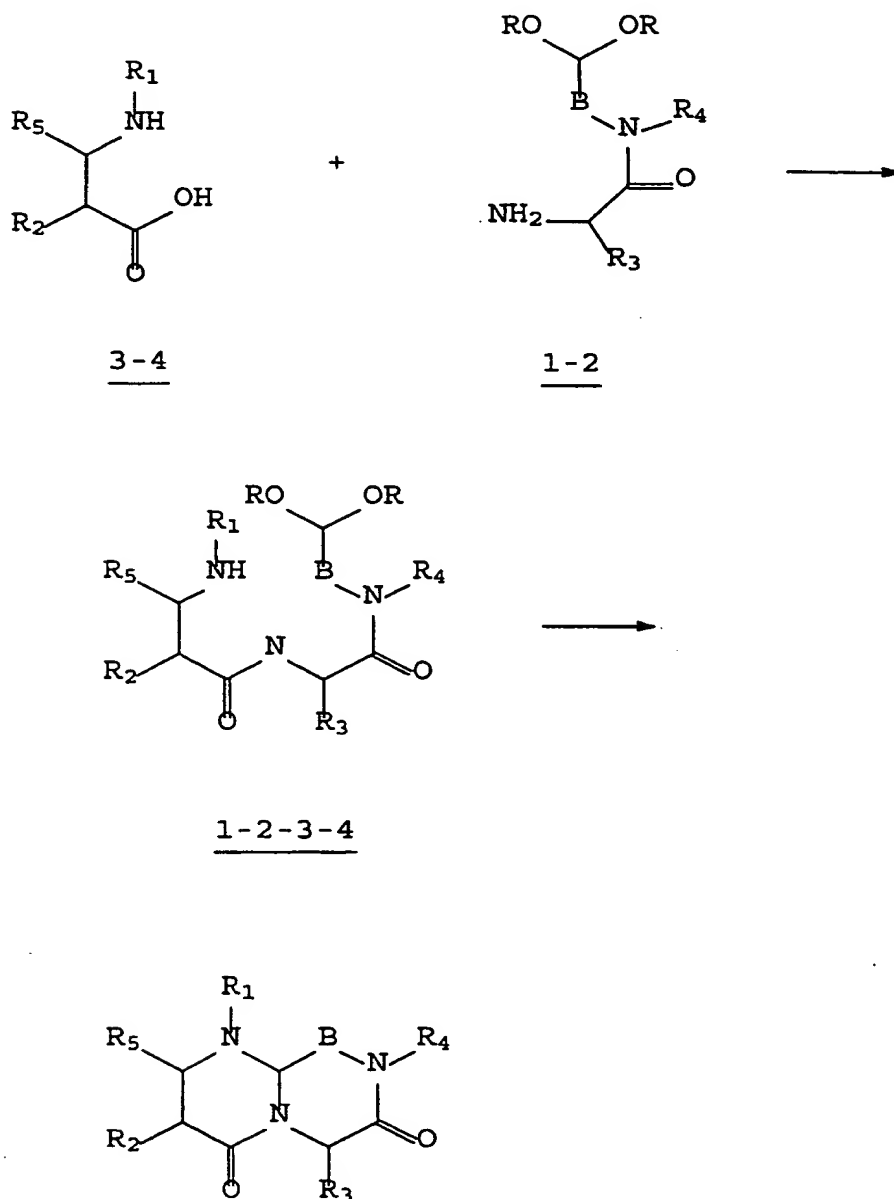
In the case where n of structure (I) above is 1 or 2, an intermediate of the following structure 3-4' can be made as follows:

5



wherein A is  $-(\text{CHR}')_n-$ . Intermediate 3-4' may then be employed in place of intermediate 3-4 in the following reactions to yield a reverse-turn mimetic of this invention having structure (I').

Coupling of the combined intermediates 1-2 and 3-4 provides intermediate 1-2-3-4 which, upon cyclization, yield the reverse-turn mimetic (I') as illustrated below:



(I') where  $n=0$

The syntheses of representative component pieces of this invention are described in Example 1. The



syntheses of representative combined first-second and third-fourth intermediates are described in Examples 2 and 3, respectively. The coupling of these intermediates to form a representative combined first-second-third-fourth intermediate is described in Example 4. The cyclization of this intermediate to form a representative reverse-turn mimetic is described in Example 5.

In a preferred embodiment, the reverse-turn mimetic of structure (Ia') may be made according to the reaction scheme set forth in Figure 2.

The reverse-turn mimetics of structures (I'') through (I''''') may be made by techniques analogous to the modular component synthesis disclosed above, but with appropriate modifications to the component pieces. More specifically, the reverse-turn mimetics of structures (I'') through (I''''') may be made by the reaction schemes set forth in Figures 3-7. In particular, the reverse-turn mimetics of structures (Ib''), (Ia'''), (Ia'''), (Ia''''') and (Ib''''') may be made by the representative reaction schemes set forth in Figures 3, 4, 5, 6 and 7, respectively.

As mentioned above, the reverse-turn mimetics of the present invention are useful as bioactive agents, such as diagnostic, prophylactic, and therapeutic agents. The opiate receptor binding activity of representative reverse-turn mimetics is presented in Example 9. In this example, the reverse-turn mimetics of this invention were found to effectively inhibit the binding of a radiolabeled enkephalin derivative to the  $\delta$  and  $\mu$  opiate receptors. The data demonstrates the utility of these reverse-turn mimetics as receptor antagonists and as potential analgesic agents.

In another aspect of this invention, libraries containing reverse-turn mimetics of the present invention are disclosed. Once assembled, the libraries of the present invention may be screened to identify individual members having bioactivity. Such screening of the

libraries for bioactive members may involve, for example, evaluating the binding activity of the members of the library or evaluating the effect the library members have on a functional assay. Screening is normally accomplished  
5 by contacting the library members (or a subset of library members) with a target of interest, such as, for example, an antibody, enzyme, receptor or cell line. Library members which are capable of interacting with the target of interest are referred to herein as "bioactive library  
10 members" or "bioactive mimetics". For example, a bioactive mimetic may be a library member which is capable of binding to an antibody or receptor, which is capable of inhibiting an enzyme, or which is capable of eliciting or antagonizing a functional response associated, for  
15 example, with a cell line. In other words, the screening of the libraries of the present invention determines which library members are capable of interacting with one or more biological targets of interest. Furthermore, when interaction does occur, the bioactive mimetic (or  
20 mimetics) may then be identified from the library members. The identification of a single (or limited number) of bioactive mimetic(s) from the library yields reverse-turn mimetics which are themselves biologically active, and thus useful as diagnostic, prophylactic or therapeutic  
25 agents, and may further be used to significantly advance identification of lead compounds in these fields.

Synthesis of the peptide mimetics of the library of the present invention may be accomplished using known peptide synthesis techniques, in combination with the  
30 first, second and third component pieces of this invention. More specifically, any amino acid sequence may be added to the N-terminal and/or C-terminal of the conformationally constrained reverse-turn mimetic. To this end, the mimetics may be synthesized on a solid support  
35 (such as PAM resin) by known techniques (see, e.g., John M. Stewart and Janis D. Young, *Solid Phase Peptide*

Synthesis, 1984, Pierce Chemical Comp., Rockford, Illinois) or on a silyl-linked resin by alcohol attachment (see Randolph et al., *J. Am Chem. Soc.* 117:5712-14, 1995).

In addition, a combination of both solution and  
5 solid phase synthesis techniques may be utilized to synthesize the peptide mimetics of this invention. For example, a solid support may be utilized to synthesize the linear peptide sequence up to the point that the conformationally constrained reverse-turn is added to the  
10 sequence. A suitable conformationally constrained reverse-turn mimetic which has been previously synthesized by solution synthesis techniques may then be added as the next "amino acid" to the solid phase synthesis (i.e., the conformationally constrained reverse-turn mimetic, which  
15 has both an N-terminus and a C-terminus, may be utilized as the next amino acid to be added to the linear peptide). Upon incorporation of the conformationally constrained reverse-turn mimetic into the sequence, additional amino acids may then be added to complete the peptide bound to  
20 the solid support. Alternatively, the linear N-terminus and C-terminus protected peptide sequences may be synthesized on a solid support, removed from the support, and then coupled to the conformationally constrained reverse-turn mimetic in solution using known solution  
25 coupling techniques.

In another aspect of this invention, methods for constructing the libraries are disclosed. Traditional combinatorial chemistry techniques (see, e.g., Gallop et al., *J. Med. Chem.* 37:1233-1251, 1994) permit a vast  
30 number of compounds to be rapidly prepared by the sequential combination of reagents to a basic molecular scaffold. Combinatorial techniques have been used to construct peptide libraries derived from the naturally occurring amino acids. For example, by taking 20 mixtures  
35 of 20 suitably protected and different amino acids and coupling each with one of the 20 amino acids, a library of

400 (i.e.,  $20^2$ ) dipeptides is created. Repeating the procedure seven times results in the preparation of a peptide library comprised of about 26 billion (i.e.,  $20^8$ ) octapeptides.

5 In a further aspect of this invention, methods for screening the libraries for bioactivity and isolating bioactive library members are disclosed. The libraries of the present invention may be screened for bioactivity by a variety of techniques and methods. Generally, the  
10 screening assay may be performed by (1) contacting a library with a biological target of interest, such as a receptor, and allowing binding to occur between the mimetics of the library and the target, and (2) detecting the binding event by an appropriate assay, such as by the  
15 colorimetric assay disclosed by Lam et al. (*Nature* 354:82-84, 1991) or Griminski et al. (*Biotechnology* 12:1008-1011, 1994) (both of which are incorporated herein by reference). In a preferred embodiment, the library members are in solution and the target is immobilized on a  
20 solid phase. Alternatively, the library may be immobilized on a solid phase and may be probed by contacting it with the target in solution.

The following examples are provided for purposes  
25 of illustration, not limitation.

## EXAMPLES

### Example 1

#### Synthesis of Component Pieces

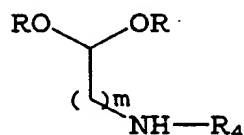
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In this example, the synthesis of representative component pieces which may be combined to form the reverse-turn mimetics of the present invention is disclosed.

35

A. Representative First Component Pieces

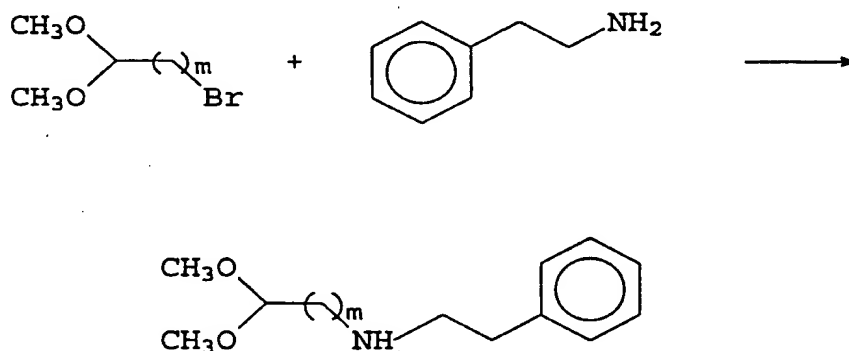
A first component piece having the following structure 1 was utilized:



1

where  $\text{R}_4$  is as defined above, and R represents a protective group suitable for use in peptide synthesis. Suitable R groups include alkyl groups and, in a preferred embodiment, R is a methyl group.

Generally, the first component piece is prepared by N-alkylation of an amine with a dialkylacetal of a 2-haloethanal. The synthesis of a representative first component piece from phenethylamine and the dimethylacetal of 2-bromoethanal is depicted schematically below.



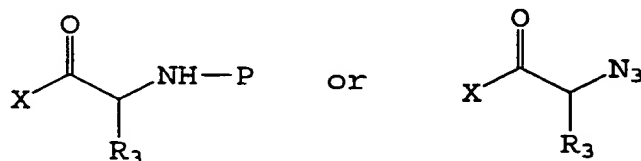
1a

In the procedure, 24 ml (3.43 ml, 20.3 mmol) of bromide and 2.8 ml (2.71 g, 22.3 mmol) phenethylamine was added 40 ml freshly distilled THF in a 150 ml argon

charged round-bottom flask equipped with a reflux condenser. The reaction was heated at a gentle reflux for 24 hours, then volatiles were removed under reduced pressure and the residue was dissolved in 200 ml dichloromethane. The organic layer was washed with 2 x 100 ml sat. aq. sodium bicarbonate, sat. aq. sodium chloride, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure and the residue dried for 3 hrs. under high vacuum to yield 3.5 g (83%) first component piece 1a (m=1) as a light brown oil used without further purification.

B. Representative Second Component Pieces

A representative second component piece of this invention is a reactive N-protected amino acid having an activated carboxylic acid group, or an azido derivative of an amino acid, as represented by the following structure 2:



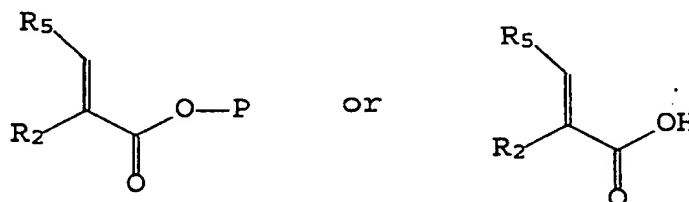
2

where  $\text{R}_3$  is as defined above, P is an amino protective group suitable for use in peptide synthesis, and X represents the leaving group of the activated carboxylic acid group. Preferred protective groups include t-butyl dimethylsilyl (TBDMS), BOC, FMOC, and Alloc (allyloxycarbonyl). N-Protected amino acids are commercially available. For example, FMOC amino acids are available from a variety of sources. The conversion of these compounds to the second component pieces of this invention may be readily achieved by activation of the carboxylic acid group of the N-protected amino acid.

Suitable activated carboxylic acid groups include acid halides where X is a halide such as chloride or bromide, acid anhydrides where X is an acyl group such as acetyl, reactive esters such as an N-hydroxysuccinimide esters and p-nitrophenyl esters, and other activated intermediates such as the active intermediate formed in a coupling reaction using a carbodiimide such as dicyclohexylcarbodiimide (DCC). Similarly, the corresponding azido derivative may be prepared by known techniques. In a preferred embodiment, X is hydroxyl for HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) coupling, or is fluorine for silicon mediated coupling.

15 C. Representative Third Component Pieces

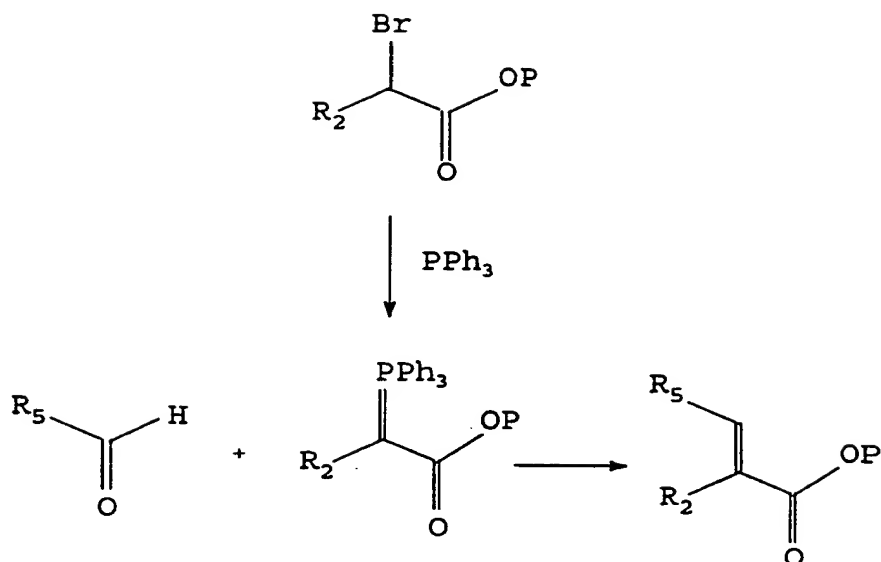
A representative third component piece of this invention is an  $\alpha,\beta$ -unsaturated carboxylic acid or derivative thereof having the following structure 3:



3

20 where  $R_2$  and  $R_5$  are as defined above, and P is a carboxylic acid protective group such as a methyl or t-butyl group. Such third component pieces may be obtained commercially, or synthesized from the commercially available aldehyde and the appropriate phosphorusylide according to the following reaction scheme:

30

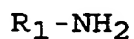


(see, Wadsworth and Emmons, *Org. Syn.* 45:44, 1965).

#### 5 D. Representative Fourth Component Pieces

A representative fourth component piece of this invention is a primary amine having the following structure 4:

10



4

where  $\text{R}_1$  is as defined above. Suitable fourth component pieces are commercially available from a variety of sources. Alternatively, the fourth component pieces may be readily prepared by standard organic synthetic techniques commonly utilized for the synthesis of primary amines.

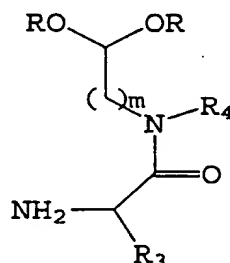
20



Example 2Combined First-Second Intermediates: The Coupling of  
First and Second Component Pieces

5       The coupling of the component pieces to produce the  
reverse-turn mimetics of the present invention generally  
involve the formation of amide bonds. The amide bonds  
which link the pieces may be formed by standard synthetic  
peptide techniques and may be performed by either liquid  
10 or solid phase synthesis.

The coupling of the first and second component pieces  
provides, after deprotection, a combined first-second  
intermediate having the following structure 1-2:

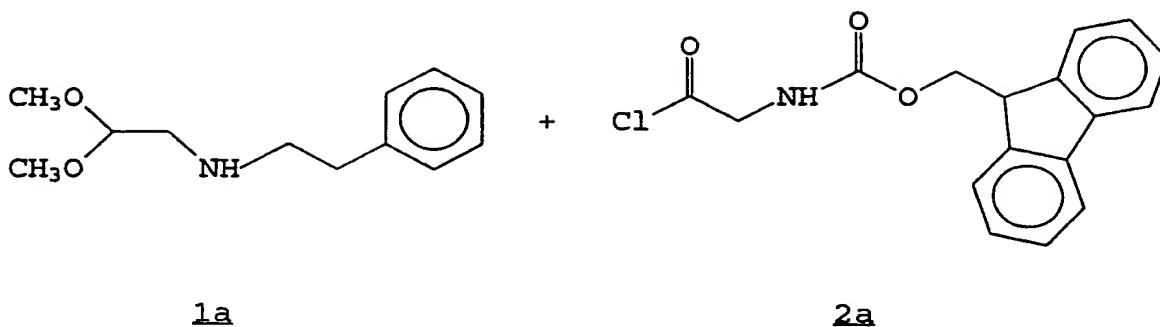
1-2

15       where R, R<sub>3</sub>, and R<sub>4</sub> are as described above (in this  
example, R" of structure (I') is/are hydrogen).

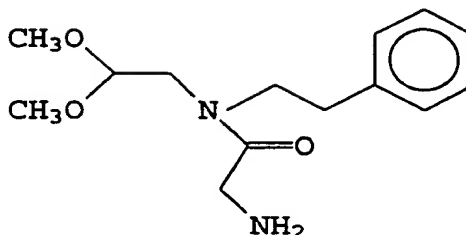
The preparation of a combined first-second  
intermediate is accomplished by amide bond formation  
20 between the amine of a first component piece 1 and the  
activated carboxylic acid group of a second component  
piece 2 followed by N-deprotection. The synthesis of a  
representative combined first-second intermediate is  
depicted schematically below.

25

32



1) AgCN  
2) DEA



1-2a

In the procedure, to 650 mg (3.17 mmol) first component piece 1a prepared as described in Example 1A and 1 g (3.17 mmol) Fmoc-glycine chloride, 2a, 10 ml freshly distilled benzene in a 25 ml argon charged round bottom flask was added 937 mg (7 mmol) silver cyanide (AgCN), and the resulting reaction mixture was stirred vigorously for 48 hrs. The reaction was diluted to 25 ml w/ethyl acetate and filtered through a Celite plug. Volatiles were removed under reduced pressure and the residue was chromatographed using 20:80 ethyl acetate:hexane as the mobile phase over flash grade silica gel to yield 1.1 g (71%) of an amorphous solid.

To 400 mg (0.82 mmol) of the amorphous solid in 5 ml acetonitrile was added 1 ml diethylamine (DEA) dropwise and the resulting reaction mixture was stirred at room

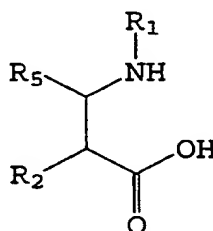
temperature for 2 hrs. The volatiles were removed under reduced pressure and the residue was chromatographed using 5% methanol saturated with ammonia 95% dichloromethane as the mobile phase over flash grade silica gel to yield 207 mg (95%) of a combined first-second intermediate, 1-2a, as a thick colorless oil.

### Example 3

#### Combined Third-Fourth Intermediates: The Coupling of 10 Third and Fourth Component Pieces

The coupling of a third component piece with a fourth component piece provides a combined third-fourth intermediate. The combined third-fourth component piece  
15 is produced by amine bond formation resulting from the conjugate addition of the amine group of a fourth component piece 4 to the  $\alpha,\beta$ -unsaturated carbonyl group of a third component piece 3.

The coupling of third and fourth component pieces  
20 provides, after deprotection, a combined third-fourth intermediate having the following structure 3-4:

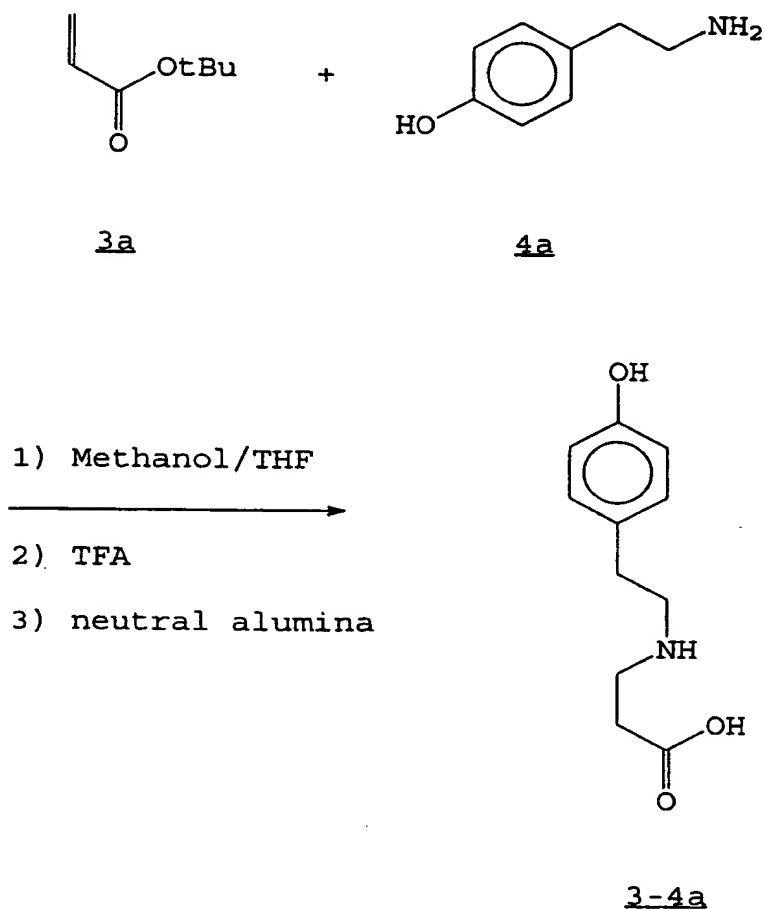


3-4

where R<sub>1</sub>, R<sub>2</sub>, and R<sub>5</sub> are as described above (in this  
25 example, n of structure (I') is 0).

The preparation of a combined third-fourth intermediate is accomplished by amine bond formation between the primary amino group of a fourth component piece 4 and  $\alpha,\beta$ -unsaturated carbonyl group of a third

component piece 3 followed by O-deprotection. The synthesis of a representative combined third-fourth intermediate is depicted schematically below.



5

In the procedure, to 5 g of tyramine suspended in 40 ml freshly distilled tetrahydrofuran (THF) in an argon charged, 250 ml round-bottom flask was added methanol sufficient to dissolve the suspension. To the resulting solution was added 5.3 ml (4.67 g, 36.4 mmol) of t-butylacrylate dropwise over the course of 5 min, and the resulting reaction mixture was stirred overnight at room temperature. An additional 2 ml of t-butylacrylate was added to consume the remaining starting material and the reaction was stirred an additional 4 hrs. Volatiles were removed under reduced pressure and the residue was

chromatographed using 95:5 dichloromethane:ammonia saturated methanol:NH<sub>3</sub>/MeOH as the mobile phase over flash grade silica gel to yield 6.6 g (68%) of the ester, a colorless oil which solidified upon overnight refrigeration. To a solution of 1 gram (3.77 mmol) of the ester in 20 ml dichloromethane at 0°C was added 80 ml of cold trifluoroacetic acid (TFA) and the resulting reaction mixture was stirred with warming to room temperature over the course of 24 hrs. Volatiles were removed under reduced pressure to yield 950 mg of a clear oil. The end product was dissolved in 95:5 dichloromethane:methanol and slowly filtered through a pad of neutral alumina. Volatiles were removed from the filtrate to yield 750 mg of 3-4a as an amorphous solid.

15

#### Example 4

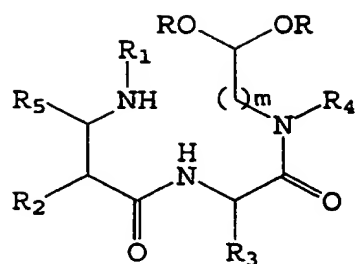
#### Combined First-Second-Third-Fourth Intermediates: The Coupling of Combined First-Second and Third-Fourth Intermediates

20

The coupling of a combined first-second intermediate with a combined third-fourth intermediate provides a combined first-second-third-fourth intermediate. The combined first-second-third-fourth intermediate is produced by amide bond formation resulting from the coupling of the amine group of a combined first-second intermediate 1-2 to the carboxylic acid group of a combined third-fourth intermediate 3-4. The combined first-second-third-fourth intermediate has the following structure 1-2-3-4:

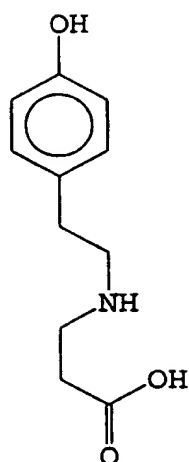
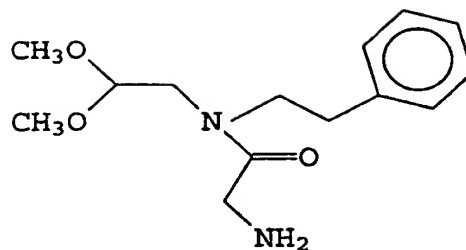
30

36

1-2-3-4

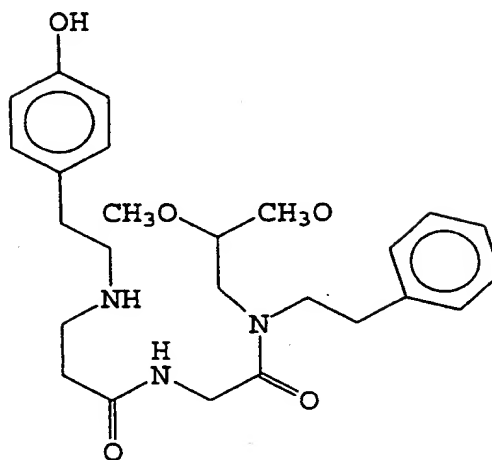
where R, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are as described above.

The synthesis of a representative combined first-second-third-fourth intermediate is depicted schematically  
5 below.

3-4a1-2a

EDC, HOBT

DMF

1-2-3-4a

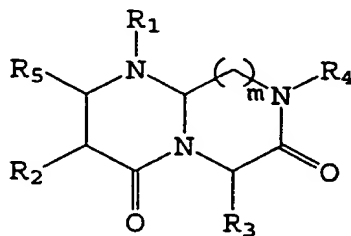
In the procedure, 212 mg (1.0 mmol) 3-4a, 270 mg (1.01 mmol) 1-2a, and 136 mg (1.01 mmol) 1-hydroxybenzotriazole hydrate (HOBT) were dissolved in 10 ml dimethylformamide (DMF) and cooled to 0°C. To this solution was added 290 mg (1.52 mmol, 1.5 eq) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and the resulting reaction mixture was stirred and warmed to room temperature over the course of 24 hours. The DMF was

removed under reduced pressure and the residue was redissolved in 200 ml ethyl acetate. The ethyl acetate layer was washed with saturated aqueous sodium bicarbonate, water, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure and the residue was chromatographed using 95:5 dichloromethane: ammonia saturated methanol as eluent over flash-grade silica gel to yield 310 mg (0.68 mm 67%) 1-2-3-4a as a thick colorless oil.

#### Example 5

#### The Synthesis of a Representative Reverse-Turn Mimetic: Cyclization of a Combined First-Second-Third-Fourth Intermediate

The cyclization of a combined first-second-third-fourth intermediate provides a reverse-turn mimetic of the present invention. The combined first-second-third-fourth intermediate 1-2-3-4 is cyclized by treatment with camphorsulfonic acid (CSA) or, in a preferred embodiment, TMSOTF (at 0°C) to provide a reverse-turn mimetic having the following structure (Ia):

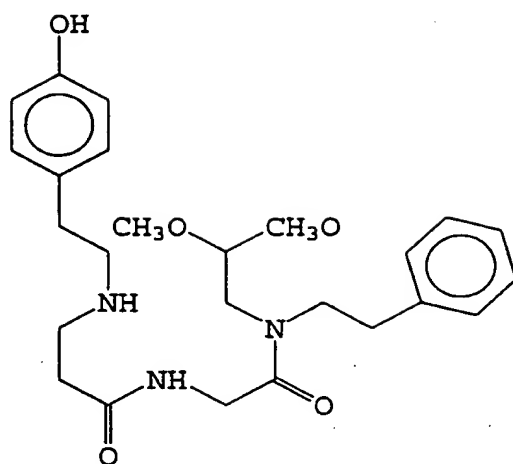
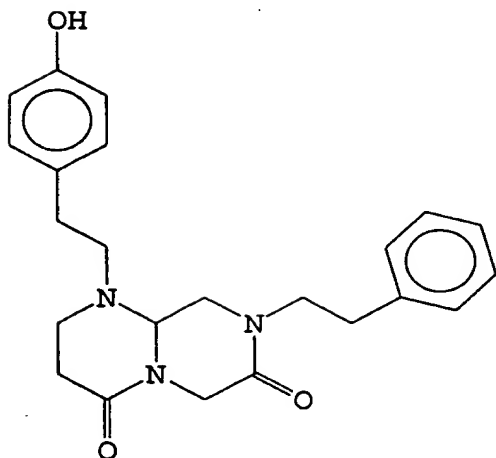
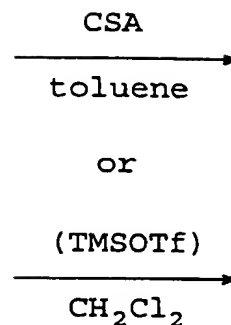


(Ia)

where R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, and R<sub>5</sub> are as described above.

The synthesis of a representative reverse-turn mimetic of the present invention is depicted schematically below.



1-2-3-4a1a

In the procedure, 0.5 g (2.4 mmol) camphorsulfonic acid (CSA) was azeotroped with 3-15 ml portions of freshly distilled toluene and dried under vacuum at 40°C for 3 hrs  
5 in a 100 ml round-bottom flask equipped with a reflux condenser. Then 20 ml of freshly distilled toluene was added and the CSA solution was heated to a vigorous reflux. To this refluxing CSA solution was added a

solution of 50 mg (0.11 mmol) 1-2-3-4a in 20 ml of freshly distilled toluene by syringe pump over the course of 1 hr. The resulting reaction mixture was refluxed for 12 hrs, cooled to room temperature and diluted to 200 ml ethylacetate. The organic layer was washed with 2-75 ml portions of saturated aqueous sodium bicarbonate, 75 ml saturated aqueous sodium chloride, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure to yield 22 mg of Ia as a glassine solid. The crude product was triturated with 50/50 diisopropyl ether:hexane to remove non-polar impurities. The solid was then dissolved in dichloromethane and filtered to remove polar impurities. The residue upon evaporation was dried in vacuo for 24 hrs.

15

#### Example 6

#### Synthesis of a Representative

#### Reverse-Turn Mimetic Salt

The reverse-turn mimetics of the present invention are nitrogen bases and may, therefore, be converted to their corresponding salts by treatment with various acids. In this example, the preparation of a representative salt of a reverse-turn mimetic is described.

The 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia, prepared as described in Example 5, was obtained by treatment of the reverse-turn mimetic with the acid in aqueous methanol. In the procedure, 5 mg (12.7  $\mu$ mol) Ia was dissolved in 3 ml of 80/20 methanol:water and cooled to 0°C. To this solution was added 2.70 mg (12.7  $\mu$ mol, 1.0 eq) 2,4 dinitrobenzoic acid, and the resulting solution stirred until it became homogenous. Volatiles were removed under reduced pressure and the residue was dried in vacuo for 24 hrs. The residue was taken up in warm water and filtered to remove insoluble impurities. The salt was then lyophilized.

Example 7  
Synthesis of a Representative  
Reverse-Turn Mimetics

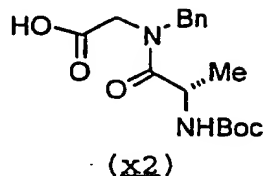
This example illustrates the synthesis of further representative reverse-turn mimetics of this invention.

Synthesis of structure (x1):



(x1)

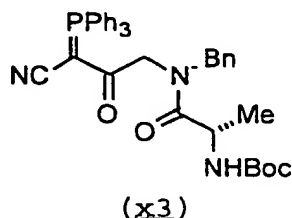
To a stirred solution of N-benzylglycine ethyl ester (1.93 g, 10 mmol) in THF (50 mL) was added Boc-Ala-OH (1.9 g, 10 mmol), followed by HOBt (1.62 g, 12 mmol) and EDCI (2.3 g, 12 mmol) at room temperature ("rt"). The resulting solution was stirred at rt for 5 hours ("h"). After dilution with EtOAc (100 mL), the solution was washed with 1N HCl (50 mL), sat. NaHCO<sub>3</sub> (50 mL), and brine (50 mL); it was dried (MgSO<sub>4</sub>), passed through a short pad of SiO<sub>2</sub>, and concentrated to give an oil in quantitative yield. TLC showed that the product was pure enough for use in the next reaction without further purification. TLC R<sub>f</sub> 0.6 (hexane: EtOAc =5:5); <sup>1</sup>H NMR (CDCl<sub>3</sub>) {the spectrum was assigned as 2:1 mixture of rotamers} δ 1.24 (two t, 3H, J=6.5 Hz), 1.35 and 1.36 (two d, 3H, J=6.5Hz), 1.42 and 1.43 (two s, 9H), 3.80 (dd, 1H, J=18Hz), 4.15 (q, 2H, J=6.5 Hz), 4.40 (dd, 1H), 4.65 (ABq, 2H, J=16.5 Hz), 4.80 (m, 1H), 5.40 (two d, 1H, J=8Hz, NH), 7.1-7.3 (m, 5H, phenyl); MS ES+ 365.1 (M+H<sup>+</sup>).

Synthesis of structure (x2):

5

To a stirred solution of 3.8 g of crude ethyl ester (x1) in THF/H<sub>2</sub>O (50/50 mL) was added LiOH·H<sub>2</sub>O (1g) at rt. After 30 min stirring at rt, the solution was washed with Et<sub>2</sub>O (50 mL) and aqueous phase was acidified by 6N HCl (pH 2), and extracted with EtOAc (3×100 mL). The combined organic extracts were dried (MgSO<sub>4</sub>), passed through a short pad of SiO<sub>2</sub>, and concentrated to provide a foam in quantitative yield. The product was used for the next reaction without further purification. <sup>1</sup>H NMR (CDCl<sub>3</sub>) {mixture of rotamers} δ 1.33 (two d, 3H, J=7 Hz), 1.41 (two s, 9H), 3.8-4.8 (set of m, 5H), 5.70 (two d, 1H, J=8Hz, NH), 7.2-7.6 (m, 5H, phenyl).

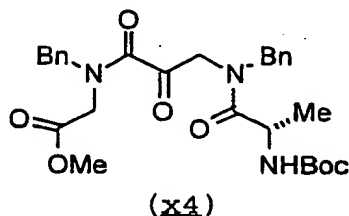
20

Synthesis of structure (x3):

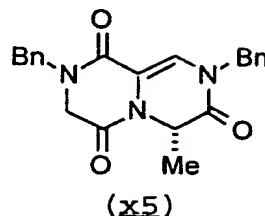
To a stirred solution of 3.4 g of acid (x2) and cyanomethylene triphenylphosphorane (4.1 g, 12 mmol) in dichloromethane (100 mL) was added sequentially DIEA (5 mL, 30 mmol), DMAP (250 mg, 2 mmol), and EDCI (2.9 g, 15 mmol) at rt. After 12 h stirring, the solution was concentrated, and the resulting residue was taken up in 1N HCl (100 mL) and extracted with EtOAc (3×100 mL). The combined extracts were washed with sat. NaHCO<sub>3</sub> (100 mL),

dried ( $\text{MgSO}_4$ ), passed through a short pad of  $\text{SiO}_2$ , and concentrated. The crude product was purified by flash chromatography (hexane:EtOAc = 50:50 to 30:70 to 20:80) to provide a foamy solid (4.40g, 71%). TLC  $R_f$  0.5 (EtOAc);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) {mixture of rotamers}  $\delta$  1.28 (two d, 3H,  $J=6.5$  Hz), 1.44 (two s, 9H), 4.2-4.7 (set of m, 5H), 5.5 (two d, 1H,  $J=8\text{Hz}$ , NH), 7.2 (m, 5H), 7.5 -7.8 (m, 15H); MS ES+  $m/z$  520.3, 620.3 ( $\text{M}+\text{H}^+$ ).

10 Synthesis of structure (x4):



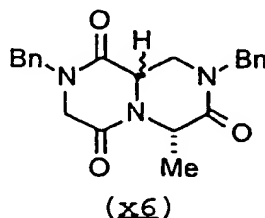
15 To a stirred solution of the phosphorane (x3) (310 mg, 0.5 mmol) in dichloromethane (5 mL) was bubbled  $\text{O}_3$  at  $-78^\circ\text{C}$  for 15 min until solution became greenish blue; TLC showed complete consumption of the starting material. After bubbling Ar to remove excess ozone from this solution, N-benzylglycine ethyl ester (100 mL) was added, and the solution was stirred at  $-78^\circ\text{C}$  for 30 min. After concentration, the residue was dissolved in EtOAc (50 mL), washed with 1N HCl (20 mL), sat.  $\text{NaHCO}_3$  (20 mL), brine (20 mL), dried ( $\text{MgSO}_4$ ), and concentrated again. The crude product was purified by flash chromatography (hexane:EtOAc = 90:10 to 80:20 to 70:30 to 60:40) to provide an oil (105 mg, 39%). TLC  $R_f$  0.42 (hexane:EtOAc = 60:40);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) {the spectrum was assigned as a 1:1 mixture of rotamers}  $\delta$  1.25 (two t, 3H,  $J=7\text{Hz}$ ), 1.31 and 1.38 (two d, 3H,  $J=7\text{Hz}$ ), 1.41 and 1.43 (two s, 9H), 3.8-4.8 (set of m, 11H), 5.5 (two d, 1H, NH), 7.2-7.4 (m, 5H). MS ES+  $m/z$  440.3, 540.3 ( $\text{M}+\text{H}^+$ ).

Synthesis of structure (x5):

5

A solution of 100 mg ketoamide (x4) (0.18 mmol) in 0.5 mL dichloromethane was treated with 0.5 mL TFA at rt for 30 min. After concentration, the residue was dissolved in MeOH (2 mL) and treated with ZnCl<sub>2</sub> (6 mg) and NaBH<sub>3</sub>CN (15 mg) at rt for overnight (13h). After concentration, the residue was taken up in sat. NaHCO<sub>3</sub> (20 mL), extracted with EtOAc (2×20 mL). The combined organic extracts were dried (MgSO<sub>4</sub>), concentrated to an oil, and purified by preparative TLC (hexane:EtOAc=60:40) to provide a glassy solid (52 mg, 77%). (The enamine proved resistant to reduction by this method.) TLC R<sub>f</sub> 0.58 (EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.41 (d, 3H, J=6.5Hz, CHCH<sub>3</sub>), 3.93 (ABq, 2H, J=18Hz, CH<sub>2</sub> in Gly), 4.46 and 4.75 (ABq, 1H each, J=14.5Hz, CH<sub>2</sub>Ph), 4.76 (ABq, 2H, J=14Hz, CH<sub>2</sub>Ph), 5.22 (q, 1H, J=7Hz, CHCH<sub>3</sub>), 6.83 (s, 1H, =CH), 7.33 (m, 10H, phenyls); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 16.63, 49.59, 49.66, 49.84, 50.98, 111.92, 119.16, 128.07, 128.22, 128.29, 128.52, 128.94, 128.97, 134.78, 134.43, 157.96, 160.67, 165.33. MS ES+ m/z 376.3 (M+H+).

25

Synthesis of structure (x6):

30

A solution of 25 mg structure (x6) (0.066 mmol) with PtO<sub>2</sub> (5 mg) in MeOH (2 mL) was stirred under H<sub>2</sub> atmosphere (20 atm) for 10 days. After concentration, the residue was purified by preparative TLC (hexane:EtOAc = 60:40 to 50:50) to yield a pale yellow oil (14 mg, 56%) with starting material (10 mg). TLC R<sub>f</sub> 0.49 (EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.14 (d, 1.5H, J=7 Hz, CHCH<sub>3</sub>), 1.52 (d, 1.5H, J=7 Hz, CHCH<sub>3</sub>), 3.2-4.8 (set of m, 10H), 7.33 (m, 10H, phenyls); MS ES+ m/z 378 (M+H). RP-HPLC analysis: C-18; A: 0.1% TFA (aq); B 0.1% TFA (CH<sub>3</sub>CN); gradient: 0-90%/40'; 254 nm tR 24.1' and 24.7' showed a 2:1 ratio.

#### Example 8

#### Synthesis of a Representative

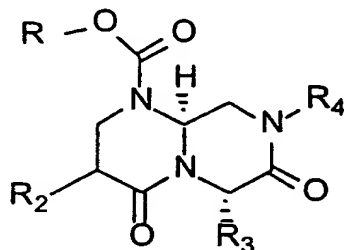
#### Reverse-Turn Mimetics

This example further illustrates the syntheses of reverse-turn mimetics of this invention. Specifically, the preparation of [4.4.0] bicyclic reverse-turn mimetics was carried out in solution phase (Method A) and on solid phase (Method B). The solid phase syntheses of these reverse-turn mimetics demonstrate that libraries containing such members may be readily prepared.

The solid phase synthesis of Method B is illustrated in Figure 8. Referring to that figure, commercially available aminomethyl resin was reacted with excess 4-bromo-2-butenic acid and DIC (diisopropylcarbodiimide) in DMF to give 4-bromo-2-butenamide resin. Substitution of the bromo group with a primary amine in DMSO gave the corresponding 4-alkylamino-2-butenamide resin. Standard peptide coupling procedures on solid phase were performed to give N-alkyloxycarbonyl- $\alpha$ -alkyl-b-alanyl- $\alpha$ -alkylglycyl-N'-alkylamino-2-butenamide resin. The reverse-turn mimetics were obtained by osmium tetroxide catalyzed periodate oxidation of the resin followed by the treatment of the resulting monocyclic product with a catalytic

amount of TFA in dichloromethane. The crude products gave a single major peak by reverse-phase HPLC analysis.

The Method A solution phase synthesis is analogous to the solid phase synthesis and was carried out essentially as illustrated in Figure 2. <sup>1</sup>H NMR was carried out on purified products of solution phase syntheses of these mimetics and spectra were assigned by a combination of COSY and ROESY experiments. All spectra were consistent with the structures indicated below, and displayed a conformation similar to a type I or type II b-turn.



	R	R2	R3	R4	Method	MS (M+1)
1.	Bn	H	Me	Me	A and B	332
2.	(CH <sub>2</sub> ) <sub>2</sub> pMeOPh	H	H	Bn	A	438
3.	(CH <sub>2</sub> ) <sub>2</sub> pMeOPh	H	H	(CH <sub>2</sub> ) <sub>2</sub> Ph	A	452
4.	(CH <sub>2</sub> ) <sub>2</sub> pHOPh	H	H	(CH <sub>2</sub> ) <sub>2</sub> Ph	A	438
5.	(CH <sub>2</sub> ) <sub>2</sub> pHOPh	H	Bn	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub>	A	494
6.	iBu	H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	iBu	A	398
7.	iBu	H	CH <sub>2</sub> CO <sub>2</sub> H	iBu	A	384
8.	Bn	Bn	Bn	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub>	A	554
9.	Bn	H	Me	Bn	B	408
10.	Bn	H	Bn	Bn	B	484
11.	Bn	H	Me	nBu	B	374
12.	Bn	H	Bn	nBu	B	449
13.	Bn	H	Me	iAmyl	B	388
14.	Bn	H	Bn	iAmyl	B	464



Example 9Activity of a Representative Reverse-Turn  
Mimetic in Opioid Receptor Binding

5

In this example, the binding activity of representative reverse-turn mimetics to the delta ( $\delta$ ) and mu ( $\mu$ ) opioid receptors is described. In these methods, the binding of the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic of structure Ia (prepared as described in Example 6), and reverse-turn mimetic 5 (prepared as described in Example 8), were evaluated in competitive radioligand binding assays.

15 A. Opiate ( $\delta$ ) Binding Activity

In this method, membranes were prepared from whole brains of male guinea pigs and equilibrated with 2 nM [ $^3$ H]DPDPE (D-pen<sup>3</sup>, D-pen<sup>5</sup>) enkephalin for 1 hour at 4°C after which test substances were added and incubated for 4 hours at 25°C. Non-specific binding was determined in the presence of 0.3  $\mu$ M naltrindole. Bound [ $^3$ H]DPDPE was separated from free radioligand by rapid filtration through glass fiber filtermats and subsequently washed 3 times. Filtermats were then counted in the LKB Betaplate to determine specifically bound [ $^3$ H]DPDPE. (See Mosberg et al., "Structural Requirements for  $\delta$  Opiate Receptor Binding," *Molec. Pharmacol.* 31:599-602, 1987.)

Table 2Effect of Reference Compounds on [<sup>3</sup>H]DPDPE Bound (2nM)

Compound	IC <sub>50</sub> (nM)	K <sub>i</sub> (nM)	Hill Coefficient
DAMGO	4,800	1,200	1.08
DPDPE	5.5	1.3	0.86
Naltrindole	0.63	0.20	0.53
U-50488	53,000	16,000	0.73

5 In this assay, the radioligand, [<sup>3</sup>H]DPDPE, was determined to have a K<sub>d</sub> = 0.65 nM with a B<sub>max</sub> = 12.6 fmol/mg protein and a specific binding of 60%. At a concentration of 10 μM, the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia was found to inhibit radioligand binding at the

10 60% level, and exhibited a K<sub>i</sub> = 1.7 ± 0.3 μM and an IC<sub>50</sub> = 6.9 ± 1.2 μM. These results are presented in Figure 1 (o) which depicts the % inhibition of radioligand binding as a function of reverse-turn mimetic Ia concentration. Also, at a concentration of 10 μM, reverse-turn mimetic 5 was

15 found to inhibit radioligand binding at the 92% level. These results demonstrate that reverse-turn mimetics Ia and 5, in particular, and the reverse-turn mimetics of the present invention, in general, effectively inhibit binding to the δ opiate receptor, and possesses analgesic activity.

20

#### B. Opiate (μ) Binding Activity

In this method, membranes were prepared from whole brains of male guinea pigs and incubated with 2 nM [<sup>3</sup>H]DAMGO (D-Ala<sup>2</sup>, N-methyl-phe<sup>4</sup>, gly-ol<sup>5</sup>)-enkephalin) for 2

25 hours at 25°C. Non-specific binding was determined in the presence of 0.5 μM DAMGO. Bound [<sup>3</sup>H]DAMGO was separated from free radioligand by rapid filtration through glass fiber filtermats and subsequently washed 3 times. Filtermats were then counted in the LKB Betaplate to

30 determine specifically bound [<sup>3</sup>H]DAMGO. (See Patricia

et al., "Pharmacological profiles of fentanyl analogs at  $\mu$ ,  $\delta$  and  $\kappa$  opiate receptors," *Eur. J. Pharmacol.* 213:219-225, 1992.)

5

Table 3Effect of Reference Compounds on [ $^3$ H]DAMGO Bound (2nM)

Compound	IC <sub>50</sub> (nM)	K <sub>i</sub> (nM)	Hill Coefficient
DAMGO	6.5	0.59	0.92
DPDPE	4.0	0.37	1.32
Fentanyl	14	1.2	0.99
Naloxone	9.3	0.76	1.09
Naltrindole	27	2.5	0.98
Norbinaltorphimine	280	26	1.13
U-50488	6.1	0.59	0.70

In this assay, the radioligand, [ $^3$ H]DAMGO, was determined to have a  $K_d = 0.27$  nM with a  $B_{max} = 8.7$  pmol/mg protein and a specific binding of 70%. At a concentration of 10  $\mu$ M, the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia inhibited radioligand binding at the 64% level, and exhibited a  $K_i = 0.64 \pm 0.08$   $\mu$ M and an  $IC_{50} = 5.4 \pm 0.7$   $\mu$ M. These results are presented in Figure 1 (•) which depicts the % inhibition of radioligand binding as a function of reverse-turn mimetic Ia concentration. Also, at a concentration of 10  $\mu$ M, reverse-turn mimetic 5 was found to inhibit radioligand binding at the 98% level. These results demonstrate that reverse-turn mimetics Ia and 5, in particular, and the reverse-turn mimetics of the present invention, in general, effectively inhibit binding to the  $\mu$  opiate receptor, and possesses analgesic activity.

25

Example 10In Vivo Activity of a Representative  
Reverse-Turn Mimetic for Analgesic Activity

5           In this example, the in vivo activity of a  
representative reverse-turn mimetic as an analgesic agent  
is presented. The 2,4-dinitrobenzoic acid salt of the  
reverse-turn mimetic of structure Ia, prepared as  
described in Example 6 (hereinafter referred to as "test  
10 compound"), was utilized in the mouse tail flick assay  
(PanLabs, Pharmascreeen Test No. 10402A). In this assay,  
the time required to elicit a tail-flick response to  
radiant heat pain stimulus in a group of mice is measured  
as the pain threshold response.

15           Groups of five (3 test groups + 1 saline control  
+ 1 morphine positive control) male ICR mice weighing 22  
( $\pm 2$ ) grams each were used. Each of these animals were  
pre-selected and elicited a tail flick response within 6-  
7.5 seconds after a focused beam of radiant heat was  
20 focused on the middle dorsal surface of the animal's tail.  
Specific amounts of the test compound (i.e., 10, 30 and  
100  $\mu$ g) were dissolved in 5 microliters (5 $\mu$ l) saline  
containing 6% DMSA and administered  
intracerebroventricularly (ICV) to each animal. A saline-  
25 only solution was used as a negative control, with an ICV  
injection of 10 $\mu$ g/5 $\mu$ l/animal of morphine serving as a  
positive control.

At one minute post-ICV injection, the groups of mice  
were measured for tail flick response, with a maximum cut-  
30 off time of 15 seconds. The mean of the response time for  
each treatment groups was calculated for a comparison  
between pre-treatment ("0 time") and 1 minute post-  
treatment 1 ("1 min."). Prolongation 1 minute post-  
treatment of over 50% ("% Prolong.") was considered  
35 significant activity. The results of this experiment are  
presented in Table 4, and demonstrate that the test

compound had significant analgesic activity (i.e., approximately 10%-15% the potency of morphine).

Table 4  
In Vivo Tail Flick Assay

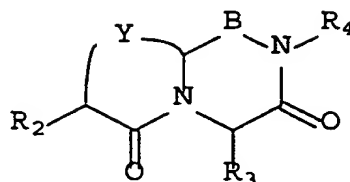
Compound	Dose/5 $\mu$ l	0 Time	1 Min.	% Prolong.
Saline	0	6.9	6.7	--
		6.9	7.5	--
		6.1	6.2	--
		6.5	6.3	--
		Avg.=6.6	Avg.=6.7	2%
Morphine	10 $\mu$ g	7.5	>15	--
		6.3	>15	--
		7.2	>15	--
		6.8	>15	--
		Avg.=7.0	Avg.>15	100%
Test Compound	100 $\mu$ g	6.5	>15	--
		6.3	>15	--
		6.5	>15	--
		6.8	>15	--
		Avg.=6.5	Avg.>15	100%
	30 $\mu$ g	6.5	>15	--
		6.7	7.2	--
		7.2	6.3	--
		6.3	>15	--
		Avg.=6.7	Avg.>15	63%
	10 $\mu$ g	6.5	7.5	--
		7.2	7.5	--
		6.9	6.7	--
		6.2	6.8	--
		Avg.=6.7	Avg.7.1	6%

It will be appreciated that, although specific embodiments of the invention have been described herein

for the purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except by the appended claims.

Claims

1. A compound having the structure:



wherein

Y is selected from  $-A-N(R_1)-CH(R')-$ ,  $-A-N(R_1)-C(=O)-$ ,  $-A-C(=O)-N(R_1)-$ ,  $-A-CH(R_1)-O-$  and  $-A-CH(R_1)-N(R')-$ ;

A is  $-(CHR')_n-$ , where  $n = 0, 1$  or  $2$ ;

B is  $-(CHR'')_m-$ , where  $m = 1, 2$  or  $3$ ;

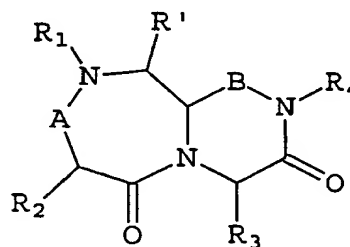
$R'$ ,  $R''$ ,  $R_2$ ,  $R_3$  and  $R_5$  are the same or different and independently selected from an amino acid side chain moiety or derivative thereof, a linker and a solid support; and

$R_1$  and  $R_4$  represent the remainder of the compound;

and

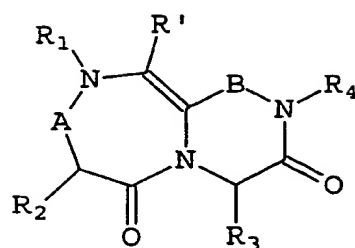
wherein any two adjacent CH groups or adjacent NH and CH groups on the fused bicyclic ring may optionally form a double bond.

2. The compound of claim 1 having the structure:

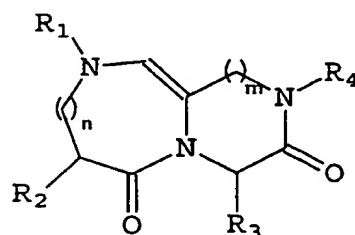


3. The compound of claim 2 having the structure:

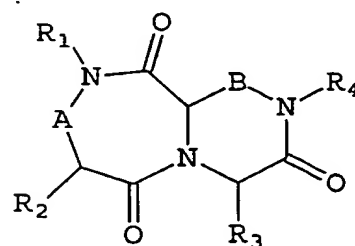
54



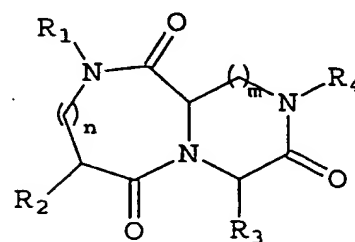
4. The compound of claim 3 having the structure:



5. The compound of claim 1 having the structure:



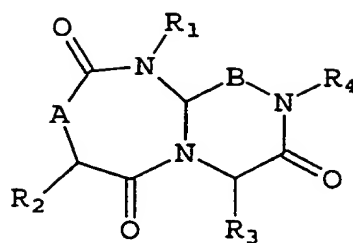
6. The compound of claim 5 having the structure:



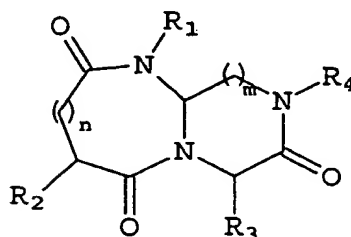
7. The compound of claim 1 having the structure:



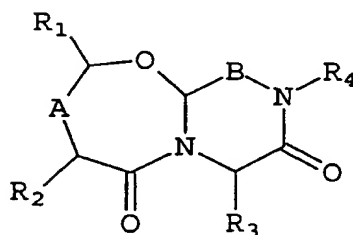
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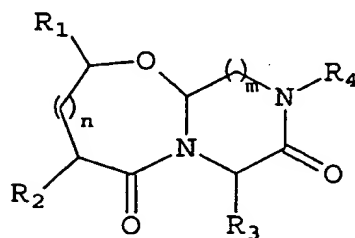
8. The compound of claim 7 having the structure:



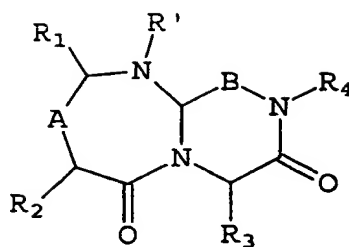
9. The compound of claim 1 having the structure:



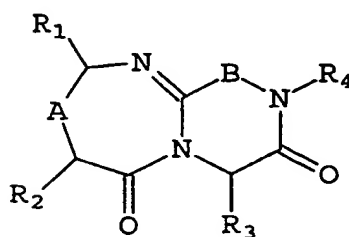
10. The compound of claim 9 having the structure:



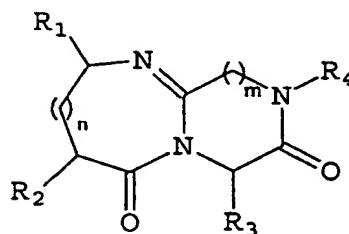
11. The compound of claim 1 having the structure:



12. The compound of claim 11 having the structure:



13. The compound of claim 12 having the structure:



14. A composition comprising a compound of claim 1 in combination with a pharmaceutically acceptable carrier or diluent.

15. A library of compounds comprising a compound of claim 1.

16. A method of identifying a biologically active compound, comprising screening the library of claim 15 to identify the biologically active compound.

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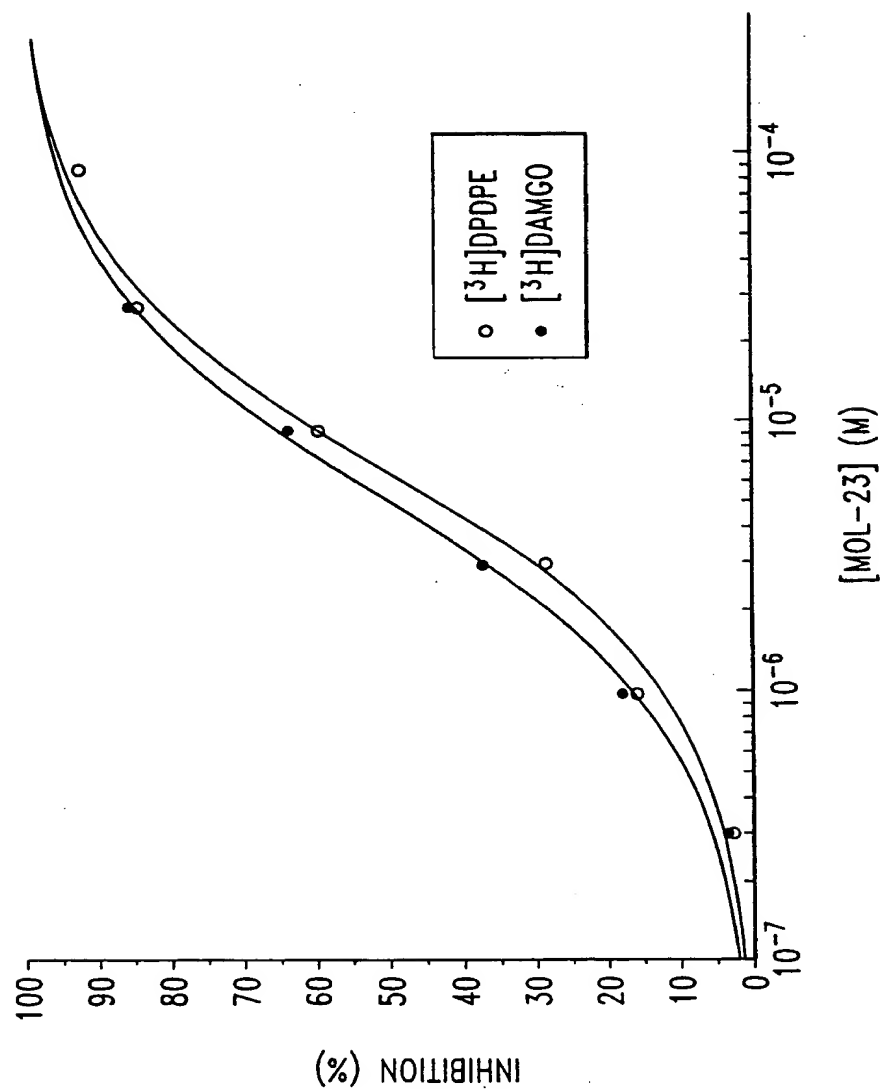


Fig. 1

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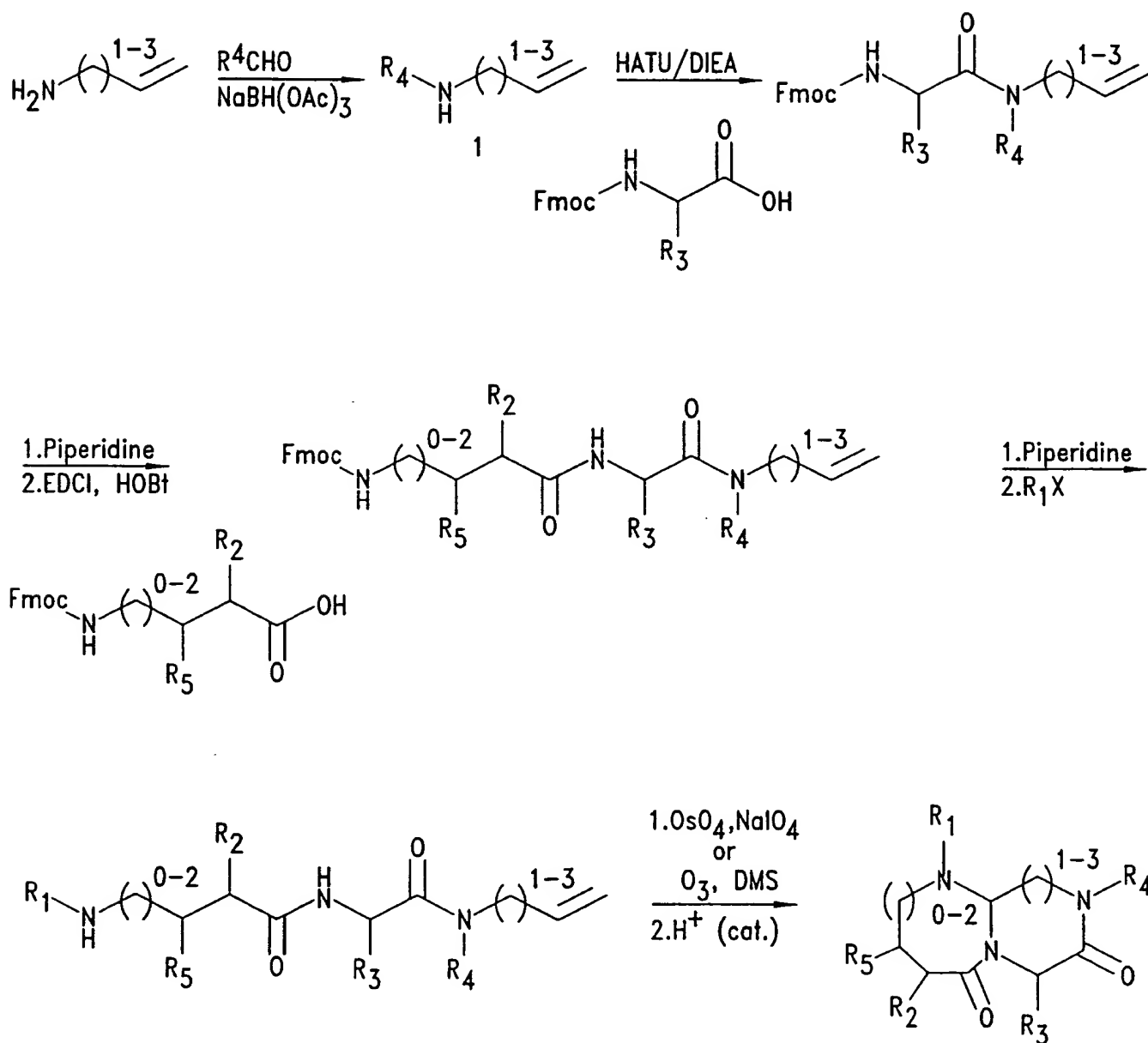


Fig. 2

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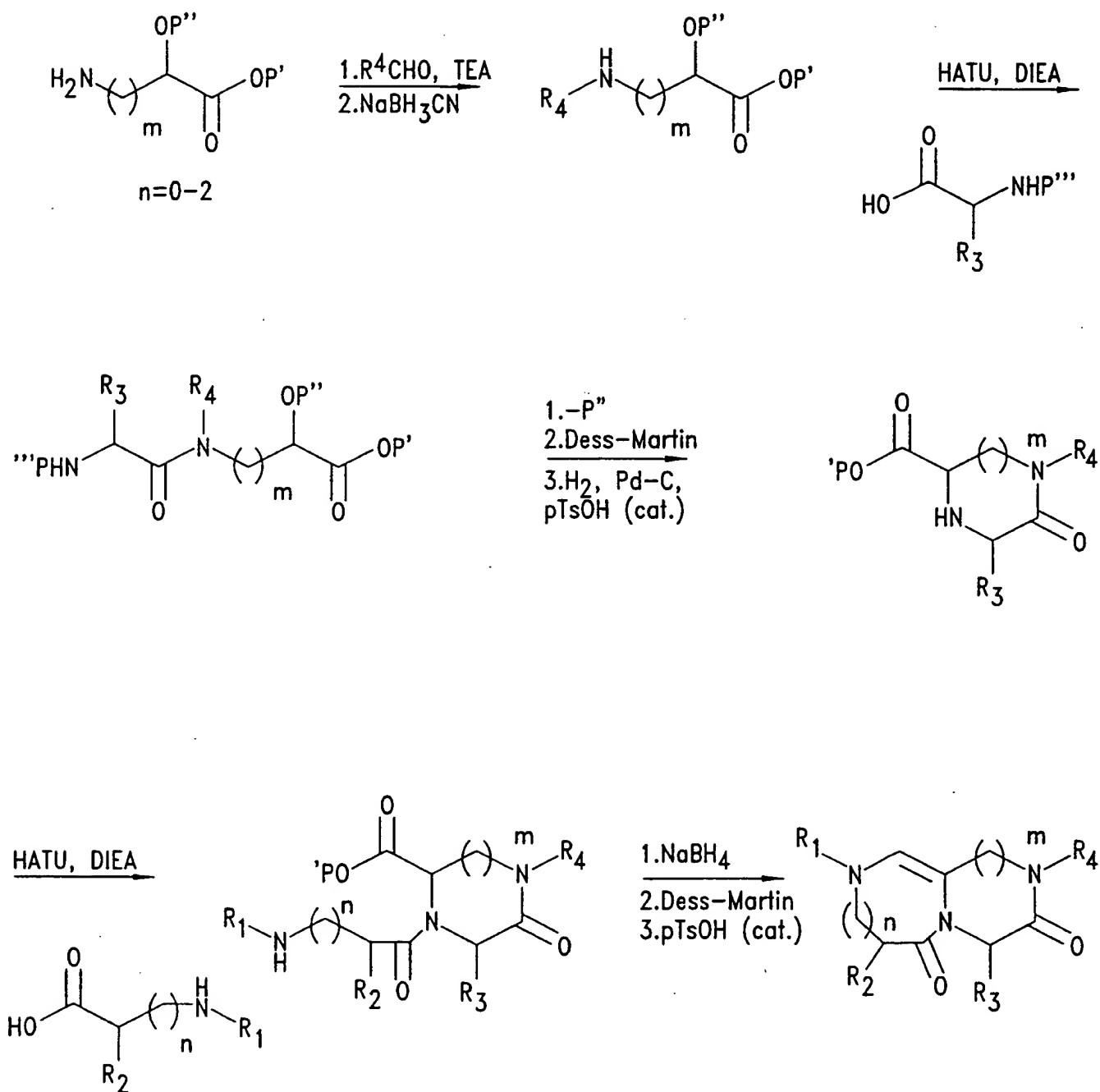


Fig. 3

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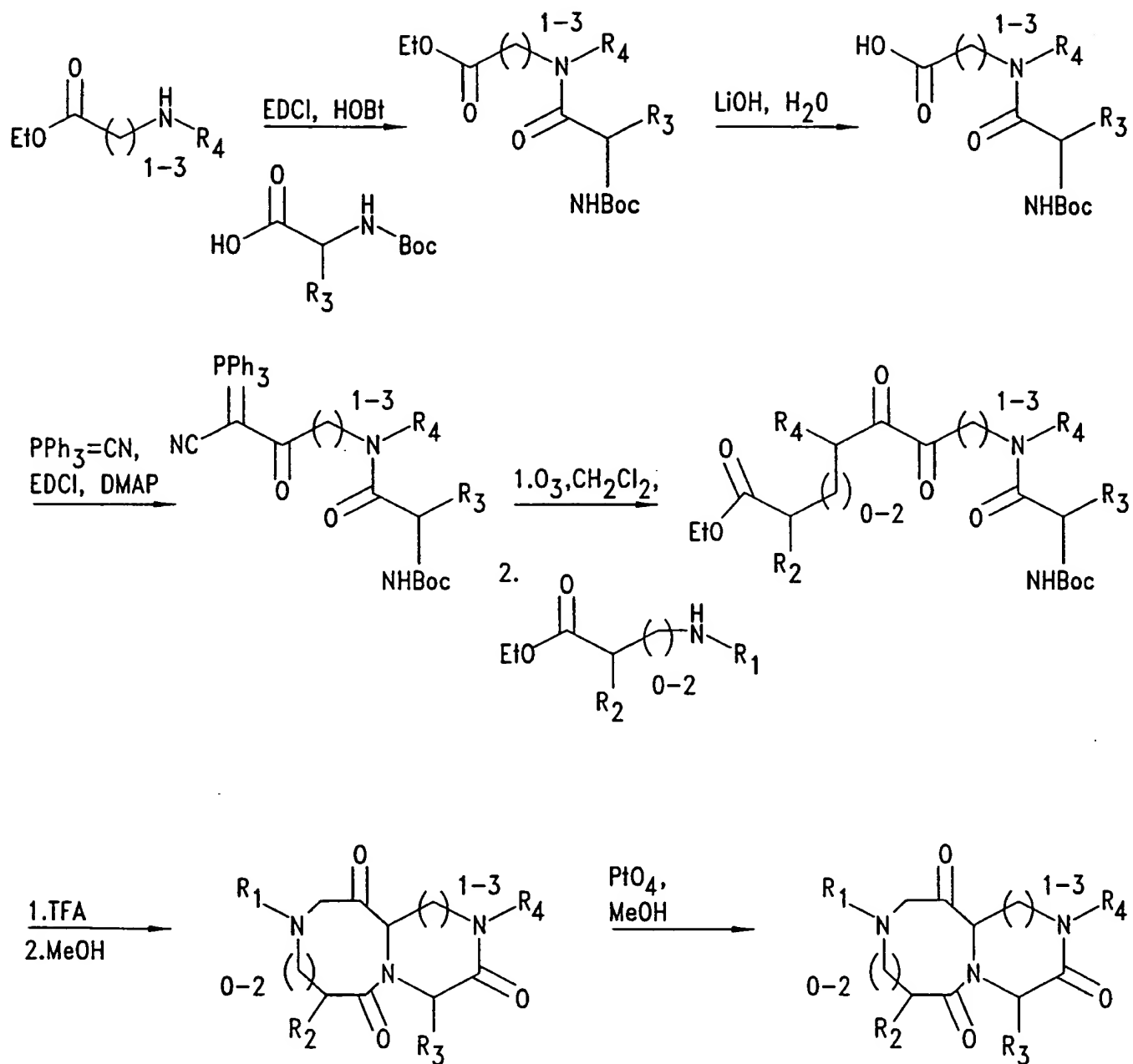


Fig. 4

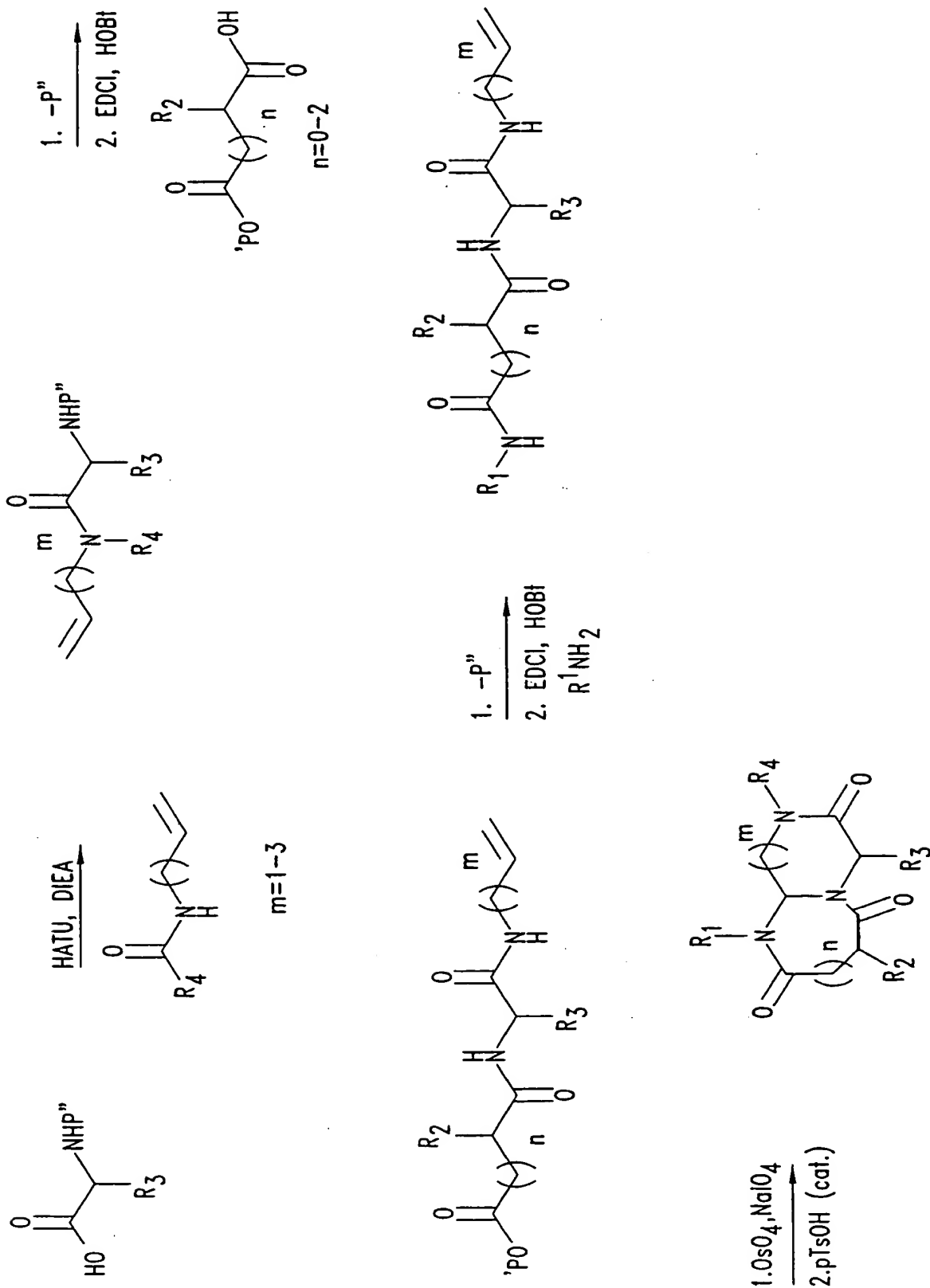


Fig. 5

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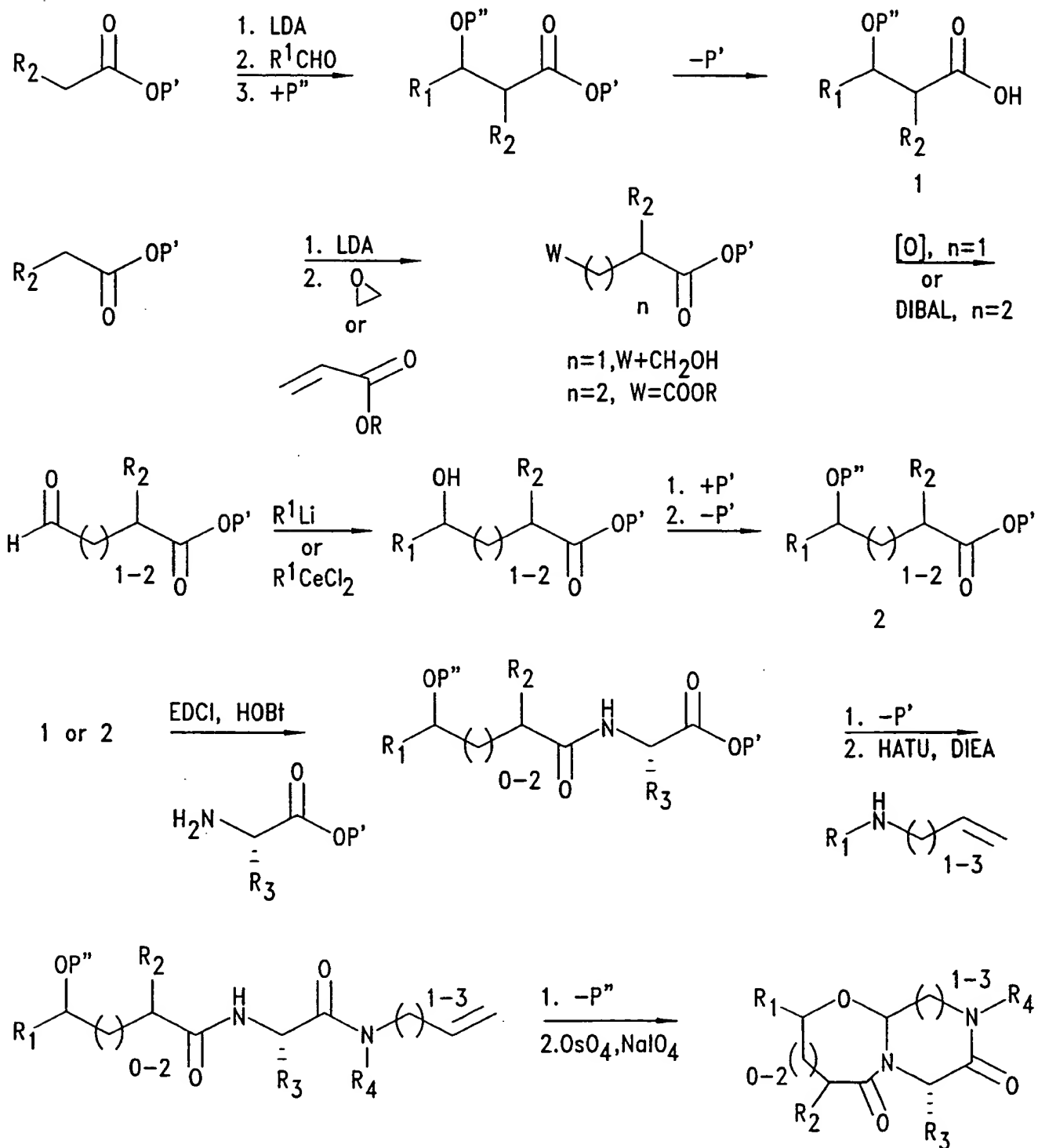


Fig. 6

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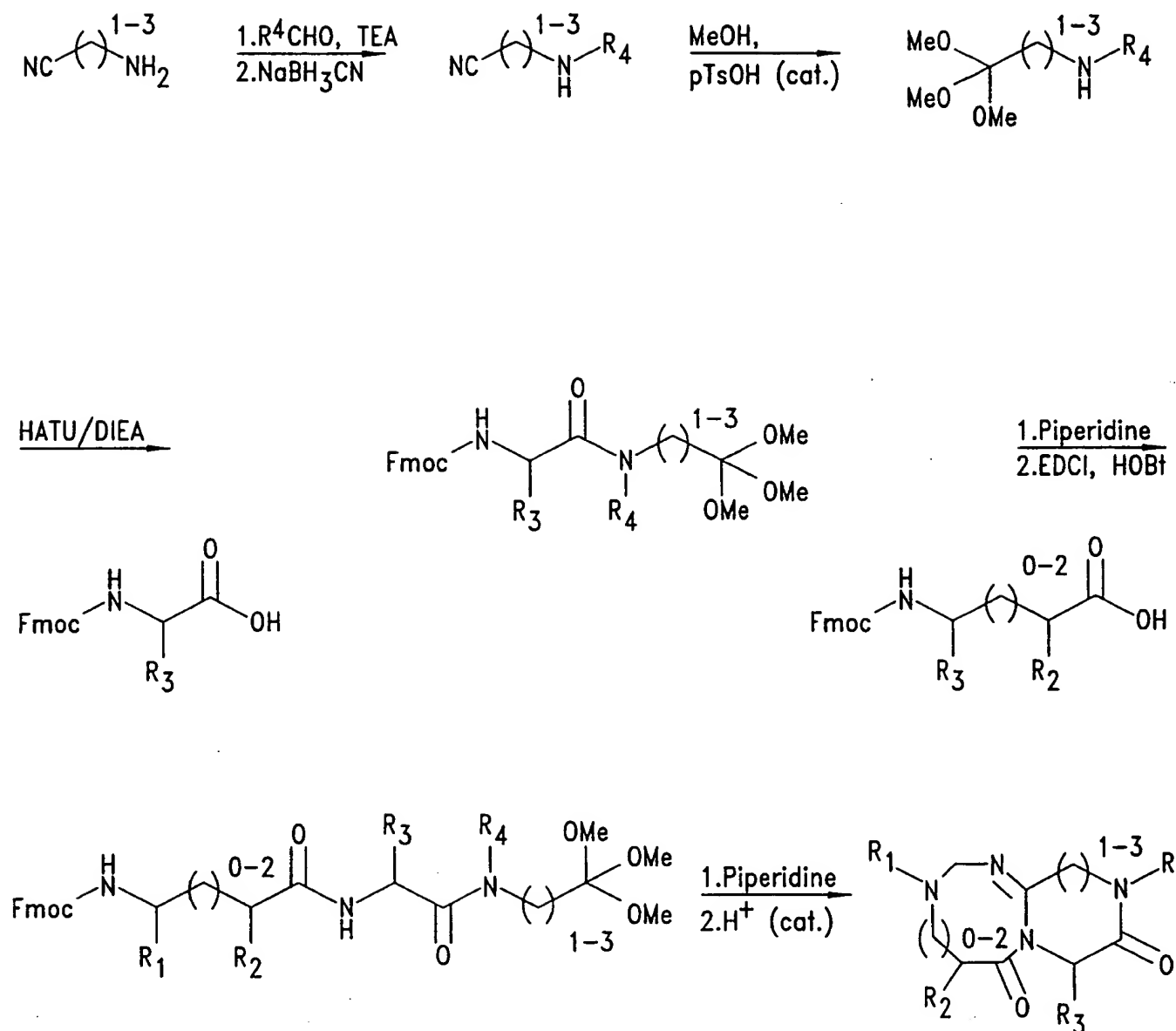


Fig. 7

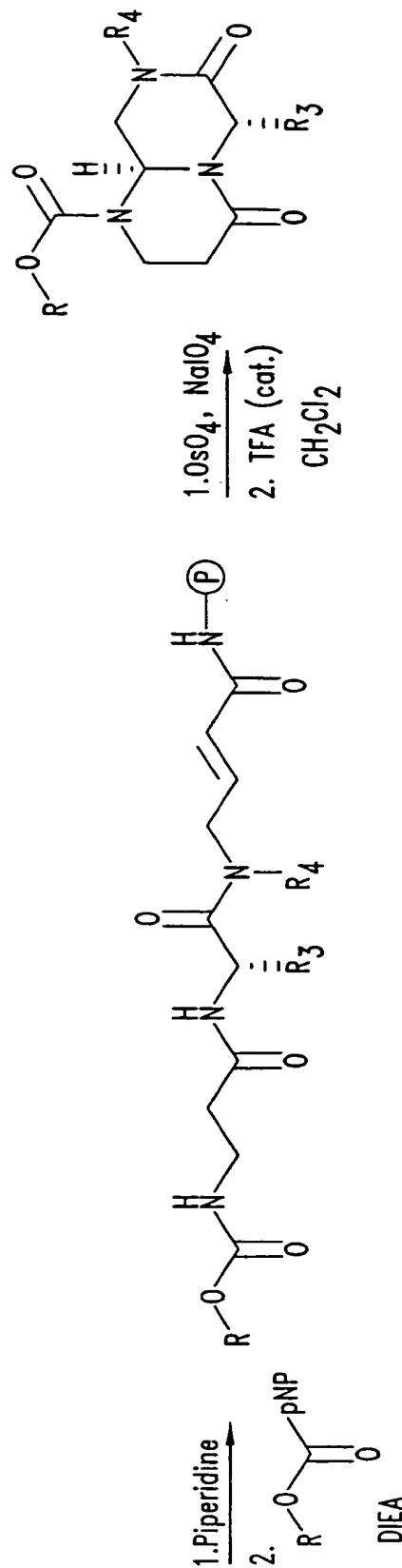
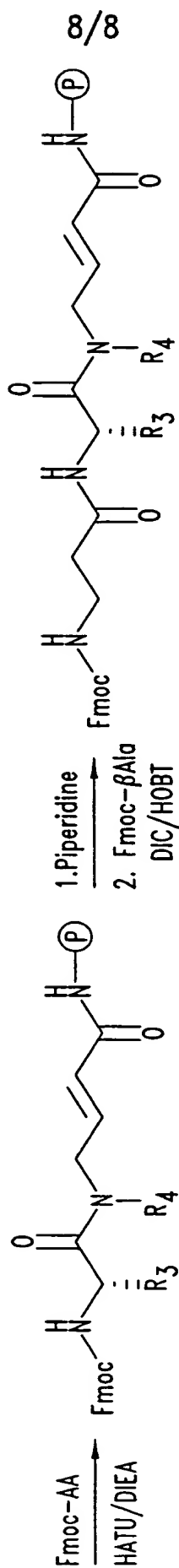
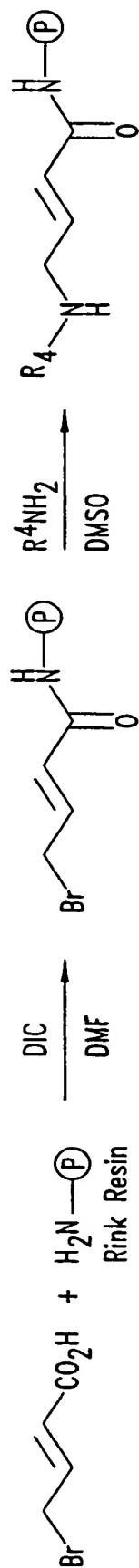


Fig. 8

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08542

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D487/04 C07D498/04 A61K31/495 C07K5/06 G01N33/50  
G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W0 94 03494 A (ILLINOIS) 17 February 1994 cited in the application see the whole document	1, 14
P, X	W0 97 15557 A (MOLECUMETICS) 1 May 1997 see the whole document	1-14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 August 1998

Date of mailing of the international search report

27/08/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Francois, J

# INTERNATIONAL SEARCH REPORT

Information on patent family members

In International Application No  
PCT/US 98/08542

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9403494 A	17-02-1994	AU 679460 B	03-07-1997
		AU 5000693 A	03-03-1994
		CA 2141447 A	17-02-1994
		EP 0656907 A	14-06-1995
		JP 7509723 T	26-10-1995
		US 5475085 A	12-12-1995
		US 5670155 A	23-09-1997
		US 5672681 A	30-09-1997
WO 9715557 A	01-05-1997	AU 7522596 A	15-05-1997